World Journal of Fish and Marine Sciences 1 (1): 01-06, 2009
ISSN 1992-0083

Effect of Garlic (*Allium sativum*) on Some Heavy Metal (Copper and Zinc) Induced Alteration in Serum Lipid Profile of *Oreochromis niloticus*

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Abstract: This study was conducted to investigate the effect of treatment with garlic oil (*Allium sativum*) on some heavy metal (copper and zinc) toxicity in *Oreochromis niloticus*. groups of Nile tilapia fed on six different diets, the concentration of CuSO4 was 300 mg/kg diet and ZnSO4 400 mg/kg diet. Garlic oil was added with concentration of 250 mg/kg diet, the experiment was extended for three months. Bioacuumulation of copper and zinc were obvious in treated fish as monitored by the significant ($P<0.01$) increase in serum and liver tissues concentrations, but they were decreased significantly after treatment with *Allium sativum*. Lipid profile like, triglyceride (TG), cholesterol, very low density lipoprotein (VLDL), low density lipoprotein (LDL) showed significant increase. But high density lipoprotein (HDL) level significantly decrease after treatment with heavy metal. Simultaneous garlic administration with copper sulfate and zinc sulfate showed improvement in serum cholesterol, LDL, HDL, VLDL, TG, Ceruloplasmin, glutathione peroxidase and Malonyldialdehyde. The results indicate that garlic (*Allium sativum*) has some beneficial effect in preventing heavy metal (copper and zinc) induced alterationi of lipid profile.

Key words: Garlic (*Allium sativum*) % Copper sulfate % Zinc sulfate % Lipid peroxidation % Serum lipid profile

INTRODUCTION

The toxicity of essential elements such as Cu and Zn tend to be more complicated than that of non essential elements such as Hg and Pb [1]. Copper which was used for different reasons in aquaculture, as for eradication of fish external parasite and as antifungal, antibacterial disease. Copper sulphate is also used as mollusciside and herbicide, but it has toxic effect on fish if the dose increased to be more than the permissible concentration[2]. Copper is bound to " globulin (" ceruloplasmin) in blood serum [3]. Also, zinc is bound to albumin and $ macroglobine in serum and is a cofactor for the protein splitting enzyme carboxypeptidse,s also, it plays a role in carbohydrate digestion by interconvert ion of pyruvate to lactate in glycolysis [4]. The minimum dietary copper requirement of channel cat fish was 5mg/kg of total cooper based on the enzymatic data [5] and there is a correlation between the metal content of water and it concentration in fish muscles [6].

Garlic administration (*Allium sativum*) has some beneficial effect in preventing heavy metal (nickel and chromium VI) induced alteration in lipid profile [7]. The most plausible mechanism that may be operative *in vivo* is the generation of reactive oxygen species (ROS) by heavy metals, which initiates lipid peroxidation, thereby causing oxidative damage to critical macromolecules like proteins, DNA as well as cell damage/ death [8,9]. For thousands of years, amazing medicinal power has been attributed to garlic. Garlic is a hardy perennial bulb, native to the Mediterranean regions of Africa and Europe. Garlic is said to lower serum cholesterol, enhance fibrinolytic activity and inhibit platelet aggregation [10,11]. There is also a report on the beneficial effects of garlic extract in controlling hyperlipidemia in animals [12]. All these effects are attributed to the presence of various organosulfur compounds, mainly allicin in garlic [13]. Garlic compounds were reported to have tremendous antioxidant property which exerts actions by scavenging ROS, enhancing cellular antioxidant enzymes and increasing glutathione in the cells [14]. The presents

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study was designed to ascertain the effect of garlic oil on heavy metal (copper sulfate and zinc sulfate) detoxication and induced alteration of serum lipid profile in *Orechromis niloticus.*

MATERIALS AND METHODS

Chemicals: Garlic oil manufactured in Germany, pharmaceutical industrialist-franconpharm GmbH, copper sulfate, zinc sulfate in the present study were obtained from El. Nasr Co., Egypt.

Experimental Fish: Nile tilapias with average body weight of 20-25 g/ fish were used in the present study. The used fish were apparently healthy and free from any abrasions or external parasites. They were acclimatized in glass aquaria of for two weeks and supplied with dechlorinated aerated tap water. The aquaria were cleaned and water was changed weekly. Dissolved oxygen was maintained at an acceptable levels (5.5-6.6 mg/l), measured by oxygen meter, water temperature was thermostatically controlled at the range of 26-28ºC, with thermostatic controlled heater and measured by thermometer and water pH was adjusted at 7.4, using pH meter.

Preparation of Experimental Diets: Six diets were prepared as shown in Table 1. The diet was pelleted trough fodder machine. The pellets were dried in a drying oven for 48 hours at 45°C, then cooled and saved in plastic bags and stored in refrigerator at 2°C during the experimental duration to avoid the nutrients deterioration.

Experimental Design: Fish were divided into 6 equal groups (each group has 10 fish) one of them served as the control group and fed on normal diet without adding heavy metal or garlic oil. Group2 fed on diet with 250mg garlic oil/kg, group3fed on diet with 400mg zinc/kg, group4 fed on diet with 300mg copper /kg, group5 fed on diet with 400mg zinc/kg and 250mg garlic oil /kg and group5 fed on diet with 300mg copper/kg and 250mg garlic oil /kg as indicated in Table 1.

Sample Collection: After the end of experiment, blood and liver tissues samples were collected from the fish, blood collected from caudal vein of the fish after fed on garlic containing diets for three months. The blood left to clot then centrifuged at 3000 r.p.m for 15 minutes. The separated serum samples were stored at -20°C for biochemical analysis. The fishes were sacrificing freshly after collection of blood samples immediately the liver tissues were taken and homogenate, used for biochemical analysis.

Determination of Copper and Zinc Bioaccumulation: Copper bioaccumulation was measured in both blood serum[15] and tissues [16] using Perkin-Elmer 2280 Atomic absorption spectrophotometer with slit 0.7.

Biochemical Analysis: Serum total cholesterol was determined according to Pearson *et al.* [17], triglycerides [18], low-density lipoprotein LDL, high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) were assayed using an enzymatic estimation kit (ERBA-diagnostics Mannheim, GmbH, Germany) [19]. Malonyl dialdehyde (MDA) were determined according to the method described by Nair and Turner [20] Malonyl dialdehyde formed in the course of lipid peroxidation was determined with 2-thiobarbituric acid (TBA). The homogenate were prepared 0.5 ml homogenate was taken without filtration and 4.5 ml of TBA reagent was added. The mixture was heated using boiling water bath for 20 min, centrifugation at 2500 r.p.m The photometric measurement was carried out at 532 nm. Ceruloplasmin activity was determined according to Owen [21]. Glutathione peroxidase (Gper) activity; was determined by measurement of the reduced glutathione substrate (GSH) remaining after the action of the enzyme, using the combined methods of Chiu *et al*. [22], with Ellmans reagent in presence of cumene hydro peroxide as a secondary substrate. The unit of enzyme activity is the amount of glutathione peroxides which consumes 1µ mol reduced glutathione / min in presence of cumene hydro peroxide.

Statistical Analysis: Data were analyzed by analysis of variance using the SAS program [23]. Duncan's multiple-range test [24] was used to verify significance of the mean differences among treatments.

RESULTS

The results showed significant ($P<0.01$)increase in Cu bioaccumulation in blood serum and liver (2.35±0.10 mg/l and 52.23±0.95 mg/kg, respectively) of Nile tilapia fed on copper supplemented diets compared to the control group (1.05±0.05 mg/l and 5.69±1.21 mg/kg, respectively) and garlic oil treated groups (1.56±0.11 mg/l and 40.95±1.33 mg/kg). As well as significant ($P<0.01$) increase in Zn bioaccumulation in blood serum and liver tissues (15.90±0.07 mg/l and 70.21±1.55 mg/kg, respectively)of

Table 1: Formulation and chemical composition of experimental diets (g/kg dry diet)

Ingredients	Group1	Group 2	Group 3	Group4	Group5	Group6
Fish meal (g/kg)	200.0	200.0	200.0	200.0	200.0	200.0
Wheat bran (g/kg)	370.0	370.0	370.0	370.0	370.0	370.0
Corn starch (g/kg)	300.0	300.0	300.0	300.0	300.0	300.0
Oile mixture (g/kg)	60.0	60.0	60.0	60.0	60.0	60.0
Vitamin premix (g/kg)	40.0	40.0	40.0	40.0	40.0	40.0
Mineral premix (g/kg)	30.0	30.0	30.0	30.0	30.0	30.0
Garlic oil (mg/kg)	0.0	250.0	0.0	0.0	250.0	250.0
Znic sulphte (mg/kg)	0.0	0.0	400.0	0.0	400.0	0.0
Copper sulphate (mg/kg)	0.0	0.0	0.0	300.0	0.0	300.0

Table 2: bioaccumulation of copper and zinc in serum and liver of Nile tilapia fed on copper, Zinc and garlic oil supplemented diets(Mean + SE)

Measurements	Group1 control	Group2 garlic oil	Group3 Zinc	Group4 Copper	Group5 Zinc and garlic	Group6 Cu and garlic
Copper in serum mg/L	1.05±0.05c	0.98±0.12c	1.08±0.07c	2.35±0.10a	0.99±0.11c	1.56±0.11b
Copper in liver mg/kg	5.69±1.21c	5.87±1.34c	5.21±1.55 c	52.23±0.95a	5.95±1.33c	40.95±1.33b
Zinc in serum mg/L	7.55±0.05c	7.33±0.12c	15.90±0.07a	7.85±0.10c	11.76±0.11b	7.76±0.11c
Zinc in liver mg/kg	20.59±1.21c	20.47±2.34c	70.21±1.55a	21.23±0.95c	54.95±1.33 b	19.95±1.33c

Means with the different letters for each parameter is significantly different at P<0.01

Table 3: pairoxidation activity in serum and liver of Nile tilapia fed on copper, Zinc and garlic oil supplemented diets(Mean + SE)

Measurements	Group1 Control	Group2 Garlic oil	Group3 Zinc	Group4 Copper	Group5 Zinc and garlic	Group6 Cu and garlic
Ceruloplasmine activity in serum U/L	105.78±5.23c	93.46±4.88c	102.77±3.26c	169.99±2.47a	106.55±3.87c	38.55±2.87b
Glutathione peroxidase activity U/g wet. Wt. In liver.	45.84±2.19d	38.56±2.88e	91.88±2.44b	123.38±3.23a	77.68±2.22c	92.68±2.22b
Malonyldialdehyde nmol MDA/g wet. Wt. In liver	25.62±1.23e	19.75±2.77f	41.22±1.44b	54.76±1.22a	30.55±2.33d	35.55±1.33c

Means with the different letters for each parameter are significantly different at P<0.01

Table 4: Lipid profile in serum of Nile tilapia fed on copper, Zinc and garlic oil supplemented diets (Mean + SE)

Measurements	Group1 Control	Group2 Garlic oil	Group3 Zinc	Group4 Copper	Group5 Zinc and garlic	Group6 Cu and garlic
Triglycerides in serum gm/dl	82.90±0.34e	80.29±0.43f	109.31±0.38b	142.83±0.27a	87.76±0.12d	93.76±0.12c
Cholesterol in serum mg/dl	74.35±0.88e	71.23±0.54f	105.45±0.79b	148.96±0.91 a	81.99±0.73d	100.99±0.73c
VLDL in serum gm/dl	22.75±0.37e	20.45±0.27f	39.35±0.38b	47.56±0.46a	26.77±0.83d	29.55±0.44c
LDL in serum gm/dl	45.64±0.72e	43.32±0.42f	66.53±0.75b	75.66±0.62a	50.44±0.45d	52.67±0.23c
HDL in serum gm/dl	52.58±0.99a	52.44±0.89a	45.66±0.29c	38.55±0.90d	48.54±0.74c	45.38±0.36c

Means with different letters for each parameter are significantly different at P<0.01

Nile tilapia fed on zinc supplemented diets compared to the control group (7.55±0.05 mg/l and 20.59±1.21 mg/kg, respectively) and garlic oil treated groups (11.76±0.11 mg/l and 54.95±1.33 mg/kg, respectively) as illustrated in Table 2.

Ceruloplasmine activity in blood serum showed significantly (P<0.01) increase in fish groups fed on Cu supplemented diets(169.99±2.47 U/l) as compared to the control group (105.78±5.23 U/l) or garlic treated group(138.55±2.87 U/l). Glutathione peroxidase activity in liver tissues was significantly high in fish groups fed on diets contained Cu or zinc (123.38±3.23 and 91.88±2.44 U/g wet.Wt, respectively) as compared to the control group (45.84±2.19 U/g wet.Wt) or garlic treated groups as illustrated in Table 3. Malonyldialdehyde nmol MDA/g wet. Wt. In liver showed significantly increase in fish groups fed on Cu or zinc supplemented diets (54.76±1.22 and 41.22±1.44 nmol MDA/g wet Wt, respectively) as compared to the control group(25.62±1.23 MDA/g wet Wt.)or garlic treated group as illustrated in Table 4.

Triglycerides in blood serum was significantly (P<0.01) increase in fish groups fed on Cu or zinc supplemented diets (142.83±0.27 and 109.31±0.38 mg/dl, respectively) as compared to the control group (82.90±0.34 mg/dl,) or garlic treated groups. Cholesterol level in blood serum was significantly (P<0.01)high in fish groups fed on diets contained Cu or zinc(148.96±0.91and, 105.45±0.79 mg/dl, respectively) as compared to the control group (74.35±0.88mg/dl)or garlic treated groups as illustrated in Table 4. Very Low density lipoproteins (VLDL) and Low density lipoproteins (LDL) showed significantly (P<0.01)increase in fish groups fed on Cu or zinc(47.56±0.46 and 39.35±0.38 mg/dl and,75.66±0.62 and 66.53±0.75mg/dl, respectively) supplemented diets compared to the control group(45.64±0.72 mg/dl)or garlic treated groups. But high density lipoprotein (HDL) showed significantly($P<0.01$) decrease in fish groups fed on Cu or zinc supplemented diets (45.66±0.29 and 38.55±0.90mg/dl, reswpectively) as compared to the control group (52.58±0.99 mg/dl)or garlic treated group Table 4.

DISCUSSION

Copper is an essential component, it is taken up by fish either from water through the gills or from the food through the gut. Copper becomes a toxic agent for fish if it is used with increased concentrations above the permissible limits. Results of the present work revealed that the increase of copper and zinc concentrations in serum and liver tissues of fish were positively correlated with a corresponding increase in their concentration in diets. Also,copper and zinc concentrations in serum and liver tissues of fish were inversely correlated with supplementation of garlic in diet. These results are in agreement with the observation that copper concentration in channel catfish liver were significantly higher in fish fed on diets contaminated with Cu than those fed copper-free diet [25]. When dietary Cu s increased, growth retardation as well as a concomitant increase of Cu in blood and bone of catfish has been taken place [26]. In the present work the efficacy of *Allium sativum* was more pronounced by the absence of poisoning signs as well as low metal content of tissues resulting from *Allium sativum* componenets interaction with copper and zinc in some way to prevent its deposition in tissues. The most plausible mechanism that may be operative *in vivo* is the generation of reactive oxygen species by heavy metals, which initiates lipid peroxidation, thereby causing oxidative damage [8, 9]. All these effects are attributed to the presence of various organosulfur compounds, mainly allicin in garlic [13]. Garlic compounds having tremendous antioxidant property which exerts actions by scavenging ROS [14].

Ceruloplasmine activity in blood serum was significantly increased in fish groups fed on Cu supplemented diets compared to compared to control or garlic treated groups. This may be due to the effect of *Allium sativum* componenets in suppressing the activity of ceruloplasmine by retarding copper absorption. But, in groups fed on zinc supplement diets there were no significant differences due to ceruloplasmin which is blue copper containing oxidase present in plasma and used in transport and delivery of copper to tissues cells [27] as reported in catfish, [28], in rain bow trout, [29] and in postsmolt Atlantic salmon (*Salmo salar*). Also, ceruloplasmin activity showed significant increase in blood serum of Nile tilapia and catfish groups under copper intoxication [30, 31] .

Glutathione peroxidase activity in liver tissues was significantly increase in fish groups fed on diets contained Cu or zinc as compared to the control or garlic treated groups . This result was previously reported [27] in catfish[26], [in rain bow trout [28] in postsmolt Atlantic salmon *Salmo salar* [29]. Glutathione peroxidase activity in liver tissues of Nile tilapia and catfish increased with the copper toxicity increase [30, 31], their activity decreased in Nile tilapia fed on diet contained vitamin C. *Allium sativum* administration has some beneficial effect in preventing heavy metal induced alteration of lipid profile [7]. The most plausible mechanism that may be operative *in vivo* is the generation of reactive oxygen species by heavy metals, which initiates lipid peroxidation, thereby causing oxidative damage [8, 9]. Garlic compounds are having tremendous antioxidant property which exerts actions by scavenging ROS, enhancing cellular antioxidant enzymes and increasing glutathione in the cells [14].

One of the end products of lipid peroxidation processes is malonyldialdehyde showed a significant increase in group fed on diet contained copper and zinc only compared to the control and treated groups, these results are in agreement with Lygren *et al.* [29] in postsmolt Atlantic salmon (*Salmo salar*) which fed on high or low dietary levels of vitamin E and copper which, showed positive effects of vitamin E on total superoxide dismutase activity in liver. Malonyldialdehyde in serum and tissues of Nile tilapia and catfish increased with induction of copper toxicity [30, 31], due to the generation of reactive oxygen species by heavy metals, which initiates lipid peroxidation [8, 9]. All these effects are attributed to the presence of various organosulfur compounds, mainly allicin in garlic [13]. Garlic compounds having tremendous antioxidant property [14].

Triglycerides in blood serum was significantly increased in fish groups fed on Cu or zinc supplemented diets compared to control or garlic treated groups (*Allium sativum*) on heavy metal induced changes in serum triglyceride (TG) level [7]. Simultaneous garlic administration with copper sulfate and zinc sulfate showed improvement in serum TG level. The rise in serum triglyceride is possibly due to hypoactivity of lipoprotein lipase in blood vessels which breaks up TG. The high TG level along with decreased absorption of fatty acids by adipose tissue is associated with a low level of HDL, insulin resistance and increased risk of atherosclerosis [20]. A significant decrease in serum TG level in all fish groups fed on garlic oil was supportive to other studies [32] and may be due to the TG-lowering effect of garlic by inhibition of fatty acid synthesis [33].

The cholesterol in blood serum was significantly high in fish groups fed on diets contained Cu or zinc compared to control or garlic treated groups. Garlic extracts decrease cholesterol in serum of rats after treatment with heavy metals[7]. In our study, high cholesterol level by copper and zinc intoxication may also be due to decreased activity of cytochrome P450 enzymes [34]. Garlic can depress the hepatic activity of lipogenic, cholesterogenic enzymes such as malic enzymes, fatty acid synthase, glucose-6-phosphate dehydrogenase [35]. High serum cholesterol level may be due to hepatic dysfunction [38, 39].

Plasma lipoproteins, very low density lipoproteins (VLDL) and low density lipoproteins (LDL) showed significantly increase in fish groups fed on Cu or zinc supplemented diets compared to control or garlic treated groups. But high density lipoprotein (HDL) showed significantly decrease in fish groups fed on Cu or zinc supplemented diets compared to control or garlic treated groups. These results agreed with the effect of garlic extract on heavy metal [7]. Heavy metals, induced rise in serum LDL, VLDL and TG and fall in serum HDL, may be due to changes in gene expression of some hepatic enzyme like HMG-CoA reductase (hydroxyl-methyl-glutaryl-CoA), which in turn depresses LDL-receptor gene expression [38, 39]. The rise in serum lipid profile may also be attributed to increased lipolysis, mediated by increased norepinephrine release which act through interference with the intracellular functions of Ca+2 in the cytoplasm [40]. All these events may lead to increased production of ROS, inducing oxidative stress resulting in metabolic dysfunction [41]. In the previous studies garlic is a hypolipidemic agent [42] that can help in decreasing the level of LDL, VLDL, TG and increasing the level of HDL. There is also report that garlic can depress the hepatic activity of lipogenic, cholesterogenic enzymes such as malic enzymes, fatty acid synthase, glucose-6-phosphate dehydrogenase [35]. In conclusion, both cooper and zinc affect the serum lipid profile and garlic oil treatment relatively improved serum lipid profile in treated fish.

REFERENCES

1. Burger, J. and M. Gochfeld, 1985. Early postnatal lead exposure: Behavioral effects in common tern chicks. J. Toxicol. Environ. Health, 16: 869-886.
2. Aaseth, J. and T. Norseth, 1986. Copper, in Freiberg L. G. F. Nordberg and V. Vouk (Eds.) Hand book on the toxicology of metals, 2nd Edn., Elsevier Science Publishers Amsterdam, pp: 233.
3. Goyer, R.A., 1986. toxic effects of metals cited in claassen, C.D, M.O. Amdur and Journal of Doull (Eds.) Casarett and Doull's toxicology: The Basic Science of poisons, McMillian Published Company. New York, pp: 582.
4. Sanstead, H.H., 1981. Zinc in human nutrition: In Bronner, F. and J.W. Coburn (Eds.). Disorders of mineral metabolism. Academic Press. Inc., New York, pp: 24-159.
5. Gatlin, D.M. and P.P. Wilson, 1986. Dietary copper requirement of fingerlings channel catfish diets. Aquaculture, 54: 277-285.
6. El-Domaity, N.A., 1987. Stress response of juvenile *Clarias lazer* elicited by copper. Compmarative Biochem. Physiol., 88 C: 259-262.
7. Amrita, D.G., S.N. Das, S.A. Dhundasi and K. K. Das, 2008. Effect of Garlic (*Allium sativum*) on Heavy Metal (Nickel II and ChromiumVI) Induced Alteration of Serum Lipid Profile in Male Albino Rats. Intl. J. Environ. Res. Public Health, 5: 147-151.
8. Das, K.K. S.N. Das and S. Dasgupta, 2001. The influence of ascorbic acid on nickel induced hepatic lipid peroxidation in rats. J. Basic Clin. Physiol. Pharmacol., 12: 187-194.
9. Doreswamy, K., B. Shrilatha, T. Rajeshkumar and Muralidhara, 2004. Nickel induced oxidative stress in testis of Mice: Evidence of DNA damage and Genotoxic effects. J. Androl., 25: 996-1003.
10. Chutani, S.K. and A. Bordia, 1981. The effect of fried versus raw garlic on fibrinolytic activity in man. Atherosclerosis, 38: 417-421.
11. Srivastava, K.C., 1984. Aqueous extraction of onion, garlic and ginger inhibit platelet aggregation and alter arachidonic acid metabolism. Biomedical Biochemistry Acta, 43: 335-336.
12. Ali, M., K.K. Al-Qattan, F. Al-Enezi, R.M. Khanafer and T. Mustafa, 2000. Effect of allicin from garlic powder on serum lipids and blood pressure in rats fed with a high cholesterol diet. Prostaglandins Leukotrine Essential Fatty Acids, 62: 253-259.
13. Augusti, K.T. and P.T. Mathew, 1974. Lipid lowering effect of allicin (Diallyl Diasulfide oxide) on long term feeding to normal rats. Experientia, 30: 468-470.

14. Borek, C., 1981. Antioxidant health effects of aged garlic extract. J. Nutr., 131: 1010-1015.
15. Makino, T. and K. Takahara, 1981. Direct determination of plasma copper and zinc in Infants by Atomic absorption with Nebulization. Clin. Chem., 27: 1445.
16. Greig, R.A., T.K. Sawyer, E.T. Lewis and M.E. Galasso, 1982. A study of metal concentration in relation to gill olor and pathology in rock carp. Arch Environ. Contamination Toxicol., 19: 454.
17. Pearson, S.S.S. and T.H. Mcgavack, 1953. Arapid procedure for the determination of serum cholesterol. J. Clinical Endocrinol., 25: 8-13.
18. Rice, E., 1970. Triglycerides in serum. Standard Methods Clinical Chem., 6: 215.
19. Bartis, C.A. and E. Ashwood, 1999. Text Book of Clinical Chemistry, 3rd Edn. WB Saunders Co: Philadelphia, Pennsylvania, USA, pp: 840-841.
20. Terasawa, Y., Z. Ladha, S.W. Leonard, J.D. Morrow, D. Newland and D. Sanan, 2000. Increased atherosclerosis in hyperlipidemic mice deficient in alpha-tocopherol transfer protein and vitamin E. Proc National Academic Science, U.S.A., 28: 830-834.
21. Owen, C.A., 1992. Biochemical aspects of copper: Copper proteins, ceruloplasmin and copper protein binding. Noyes Pub. Park Ridge, N. Journal, pp: 205.
22. Chiu, D.I.Y. and P.H. Stults and A.L. Tappal, 1976. Purification and preparation of rat lung soluble glutathione peroxidase. Biochemi. Biophys. Acta. 445: 558-566.
23. SAS Institute, 1989. SAS/SAT User's guide-version 6. Cary.
24. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 42-49.
25. Murai, T., J.W. Andrews and R.G.J. Smith, 1981. Effect of dietary copper on channel catfish. Aquaculture, 22: 353-357.
26. Gatlin, D.M. and P.P. Wilson, 1986. Dietary copper requirement of fingerling channel catfish. Aquaculture, 54(4): 277-285.
27. Wise, D.J., J.R. Tomasso, D.M.III. Gatlin, S.C. Bai and V.S. Blazer, 1993. Effects of dietary selenium and vitamin E on red blood cell peroxidation, glutathione peroxidase activity and macrophage superoxide anion production in channel catfish. J. Aaquatic Animal Health, 5: 177-182.
28. Bell, J.G., B.J.S. Pirie, J.W. Adron and C.B. Cowey, 1986. Some effects of selenium deficiency on glutathione peroxidase (EC1.11.1.9) activity and tissue pathology in rainbow trout (Salmo gairdneri). J. Nutr., 55: 305-311.
29. Lygren, B., K. Hamre and R. Waagboe, 1999. Effects of Dietary Pro-and Antioxidants on Some Protective Mechanisms and Health Parameters in Atlantic Salmon. J. Aquatic-Animal Health, 11: 211-221.
30. Metwally, A.A.M., 1998. Biochemical effect of some chemical pollutants on the activity of some enzymes in tissues of fresh water fish. A thesis Ph.D. Dept. of Biochemistry. Faculty of Vet. Medicine Zagazig University.
31. Metwally, M.A.A., M.A.A. Ali and Ell-Nobi, G. Ahmed, 2001. Effect of ascorbic acid (vitamin C) on copper (Cu) toxicity in Nile tilapia (*Oreochromis niloticus*). Zagazig Vet. J., 29: 77-88.
32. Mansell, P. and J.P.D. Reckless, 1991. Garlic Effects on serum lipids, blood pressure, coagulation, platelet aggregation and vasodilation. British Med. J., 303: 379-380.
33. Nwanjo, H.U. and G.O. Oze, 2007. Changes in Serum Lipid Profiles and Heart Rate in Rats Treated With Aqueous Garlic Extract. J. Nutr. Wellness, 4: 1.
34. Witmer, C., E. Faria, H.S. Park, N. Sadrieh, E. Yurkow, S. O'Connell, 1994. *In vivo* effects of chromium. Environmental Health Perspect, 102: 169-176.
35. Yu-Yan and L. Yeh Liu, 2001. Cholesterol-lowering effect of garlic extracts and organ sulfur compounds: Human and animal studies. J. Nutr., 131: 9895-9935.
36. Banerjee, S.K., P.K. Mukherjee and S.K. Maulik, 2003. Garlic as an antioxidant: the good, the bad and the ugly. Phytotherapy Res., 17: 97-106.
37. Ichikawa, M., J. Yoshida, N. Ide, T. Sasaoka, H. Yamaguchi and K. Ono, 2006. Tetrahydro-$-carboline derivatives in aged garlic extract show antioxidant properties. J. Nutr., 136: 726-731.
38. Kantola, T., K.T. Kivisto and P.J. Neuvonen, 1998. Grapefruit juice greatly increases serum concentration of lovastatin and lovastatin acid. Clinical Pharmacol. Therapy, 63: 397-402.
39. Kojima, M., T. Masui, K. Nemoto, M. Degawa, 2004. Lead nitrate induced development of hypercholesterolemia in rats; Sterol independent gene regulation of hepatic enzymes responsible for cholesterol homeostasis. Toxicol. Lettrature, 154: 35-44.
40. Liu, P.S. and M.K. Lin, 1997. Biphasic effects of chromium compounds on catecholamine secretion from bovine adrenal medullary cells. Toxicology, 117: 45-53.
41. Anad, S.S., 2005. Protective effect of vitamin B6 in chromium-induced oxidative stress in liver. J. Applied Toxicol., 25: 440-443.
42. Auer, W., A. Eiber, E. Hertkorn, U. Koehrle and A. Lorenz, 1990. Hypertension and hyperlipidemia: Garlic helps in mild cases. Br. J. Clin. Prac. Supply, 69: 3-6.