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Metabolic and histological studies on the effect of garlic administration on the carnivorous fish *Chrysichthys auratus*

Mohamed B. Al-Salahy* and Abd Allah B. Mahmoud

Zoology Department, Faculty of Science, Assiut University, Assiut, Egypt

ABSTRACT

Chrysichthys auratus fish were orally administered with doses of garlic (*Allium sativum*) juice equivalent to 2g per kg body wt, as a single dose or the same dose every 48h for 5 and 11 days. The results showed a hypoglycaemia, hypolipidaemia, hypocholesterolaemia, hypotriglyceridaemia and drop in serum TFAA as well as promotion of lipogenesis and glycogenesis in white muscle in response to repeated doses of garlic. Such results may correlate with elevation of insulin release in the fish following garlic administration. Histological and biochemical studies confirmed liver glycogen depletion and liver histopathological changes including vacuolation, hypertrophy and degeneration of some hepatocytes, and dilatation and congestion of some blood vessels. Histopathological changes of the kidney were vacuolation and rupture of some renal tubules, and degeneration of the parietal cells of some Malpighian corpuscles. Garlic decreased the amount of collagenous fibres in both liver and kidney, and reduced carbohydrate content in the kidney. In spite of some cell degeneration in the liver and renal tubules, serum AST and ALT did not show any significant changes, while serum ALP was decreased. Generally, it could be concluded that garlic strongly affected carbohydrate metabolism in *Chrysichthys auratus*. Garlic may improve carbohydrate metabolism, probably lowering the dietary protein costs of the fish; the damage of garlic on both liver and kidney in this work may be attributed to the relative high dose of garlic used.

KEYWORDS: garlic, fish, liver, kidney, histology, glycogenesis, lipogenesis.

INTRODUCTION

Fish in general are severely glucose intolerant (Mommsen & Plisetskaya 1991; Wright 1992). *Chrysichthys auratus* is a carnivorous fish (Khidr 1981), and Mommsen & Plisetskaya (1991) suggested that glucose intolerance in carnivorous fish was due to a blunted peripheral response to the glucosestatic effects of insulin similar to that found in human type II diabetes mellitus rather than inadequate insulin secretion. Thus carnivorous fish are less able to metabolize carbohydrate than others (Furuichi & Yone 1981). On the other hand, a problem in manufacturing artificial diets for fish is the high protein requirement of many species, contributing to high feed costs (Anderson *et al*. 1984). This work is an attempt to use a natural dietary source like garlic to activate the pancreas to secret insulin, as reported by Chang & Jhonson (1980) and Preuss *et al*. (2001).

Garlic (*Allium sativum*) has an important dietary and medicinal role which has been pointed out for centuries. Great attention has been devoted to studying the effect of dietary garlic in humans and mammals (Chang & Jhonson 1980; Ali *et al.* 2000). However, no literature is available regarding the effect of garlic diet on fish. In mammals garlic has effects of hypolipidaemia (Lawson 1994), hypoglycaemia (Kasuga *et al*. 1999), hypotriglyceridaemia (Ali *et al.* 2000) and hypocholesterolaemia (Mirhadi *et al.* 1991; Ali *et al.* 2000).

Excess doses of garlic can cause toxicity, including anaemia and gastrointestinal problems (Banerjee *et al*. 2003). High doses of garlic powder (200 mg/kg) caused considerable cell injury in rat liver (Egen-Schwind *et al*. 1992), which was not observed at lower doses. Nakagawa *et al*. (1985) found that raw garlic juice at a dose of 5 ml/kg causes

* Address for Correspondence

death of rats due to stomach injury. Administration of garlic at a rate of 1000 mg/kg caused necrosis in the liver of rats (Banerjee *et al*. 2001). From these reports it is evident that garlic can have both beneficial and toxic effects. The main garlic components are allicin and S-allyl cysteine: allicin decomposes in stomach acids to release diallyl sulphide and diallyl disulphide (Rosen *et al*. 2001).

The objective of the present work is to explore the effects of garlic on histological structures of the liver and kidney, as well as its metabolic effects on the blood serum, liver and white muscle of the Nile carnivorous fish, *Chrysichthys auratus* .

MATERIALS AND METHODS

Healthy adult Nile fish, *Chrysichthys auratus* (Alzammar) of both sexes were caught from the Nile in Assiut Governorate and transported immediately in appropriate aquaria (40 x 80 x 60 cm, capacity 100 litres) to the laboratory. The aquaria were filled with aerated fresh water. The water temperature was 21±0.6 °C. The average body weight and body length were 45.2 ± 5.3 g and 14 ± 2.5 cm respectively. The fish were fed commercial pellets twice daily at a rate of 1% of mean body weight. The fish were acclimatized to the aquaria for one week prior to the beginning of the experiment. All fish were food-deprived for 24h before being sacrificed.

Garlic (*Allium sativum*) cloves were minced in a juicer/blender device (model no. MJ 176 NR) and thus the raw natural juice was obtained. Oral administration of diluted fresh garlic juice ten times (V/V) with distilled water to the fish was carried out by using a canula. Each dose was 2g of raw garlic juice or 20 ml of diluted garlic juice / kg / body wt.

Thirty sex fish were divided into two groups, 18 fish each. The first group was further subdivided into three subgroups. The first subgroup was administered a single dose of garlic. The second and the third fish subgroups were administered repeated doses of garlic for five (3 doses) and 11 (5 doses) days, with each repeat dose administered every 48 hr. Thus each fish of these subgroups received a total of 6 g or 10 g of garlic juice over periods of 5 and 11 days respectively. The second fish group was the control which was also subdivided into three subgroups: all fish were administered distilled water instead of garlic through the same route and according to the same protocol. The fish were dissected 24 hr after the last dose.

At the end of the experiment, blood samples from each fish were collected by suction from the caudal peduncle. The blood samples were transferred to the centrifuge tubes and allowed to clot at room temperature. Serum was then separated by centrifugation at 3000 rpm for 20 min and stored deep-frozen pending analysis. Samples of liver and white skeletal muscle (below the dorsal fin) were collected. A part of both liver and muscle were dried at 60 °C to constant weight and then ground. All these samples were kept at -20 °C pending biochemical analysis.

For histological observations, small pieces of liver and kidney from each fish multiply treated with garlic were taken immediately fixed in 10% neutral formalin or Bouin's solution. The specimens were dehydrated, cleared and embedded in paraffin. Sections were obtained at 3μm and stained with haematoxylin and eosin and Masson's trichrome stain (Drury & Wallington 1980). For detection of polysaccharides, the periodic acid Schiff's technique (PAS) was used. Control sections were incubated in human saliva at 37 °C for one hour prior to the PAS technique. The absence of stained material from these sections was taken as evidence for the presence of glycogen in section subjected to PAS reaction without prior treatment with saliva (Hotchkiss 1948).

Total serum lipids were estimated using a kit (BioAdwic) according to the method of Knight *et al*. (1972). The estimations of total lipid in liver and white muscles

were made using the same kit after the extraction of lipids by chloroform: methanol (2:1) as described by Folch *et al*. (1957). Total cholesterol and triglycerides in serum and tissue lipid extracts were determined according to the methods of Fossati & Principe (1982) and Richmond (1973) respectively. Blood glucose was determined by the method of Trinder (1969), while tissue glycogen was estimated by anthrone reagent after hydrolysis of tissues in HCl (4N) for 2h in a boiling water bath (Van-Handel 1965). Extraction of total free amino acids (TEAA) of serum and tissues was conducted in 70% ethanol followed by centrifugation. The estimation of TFAA level in the supernatant was carried out according the ninhydrin method of Rosen (1957). Serum total protein and urea were estimated according to the methods described by Henry (1964) and Patton & Crouch (1977) respectively. Serum glutamate oxaloacetate transaminase GOT (AST) and glutamate pyruvate transaminase GPT (ALT) were determined according to the method of Reitman and Frankel (1957), while alkaline phosphatase (ALP) was estimated by the method of Klin (1970).

The data were statistically analysed using SPSS ver. 9 (1998). The data are presented as means ± S.E. and statistical differences among groups were detected using Student's "t" test with the help of SPSS procedures.

RESULTS

Tables 1-3 indicate that garlic administration led to severe hypoglycaemia after all periods of the experiment accompanied by liver glycogen depletion (24h after the single dose, and 5 days after three repeated doses) and elevation of white muscle glycogen at the periods following only the repeated doses. The tables also show that garlic caused significant hypolipidaemia in response to repeated doses, accompanied by decreases in liver total lipids and a significant rise in the white muscle total lipids (11-day treatment).

Garlic diminished the serum TFAA in response to repeated doses for 11 days. This result was accompanied with a transitory rise in the liver TFAA and insignificant changes in its levels in white muscle. There were significant decreases in both serum triglyceride and cholesterol levels in response to garlic repeated doses for 5 and 11 days, while garlic increased the white-muscle triglyceride content after repeated doses for 11 days. Garlic administration led to insignificant change in the serum levels of total proteins, AST and ALT (after single and repeated doses) but it decreased the serum levels of both urea and alkaline phosphatase after repeated doses for 11 days (Table 1).

The histological structure of the liver of untreated fish differs from that of mammalian liver in the absence of a definite pattern of arrangement. The hepatocytes are polygonal in shape and its cytoplasm is acidophilic. The nuclei are centrally located and spherical in shape. There are blood vessels, and sinusoids of different sizes between the liver cells (Figs. 1, 2). The livers of fish administered garlic repeatedly for 5 days show vacuolation and hypertrophy of the hepatocytes. Hypertrophy of the hepatocytes due to fatty degeneration of accumulation of lipid droplets leads to collapse of the blood sinusoids. In addition, considerable numbers of hepatocytes are characterized by the absence of nuclei, and others exhibit pycnotic nuclei (Fig. 3). In other regions of liver sections, the hepatocytes are damaged (Fig. 4).

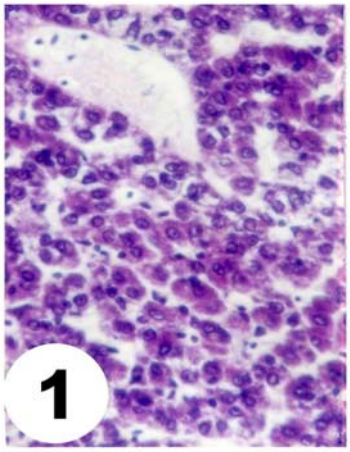

Fig 1: Photomicrograph of a section of control fish liver showing the blood vessel as well as polygonal liver cells. H & E. X400.

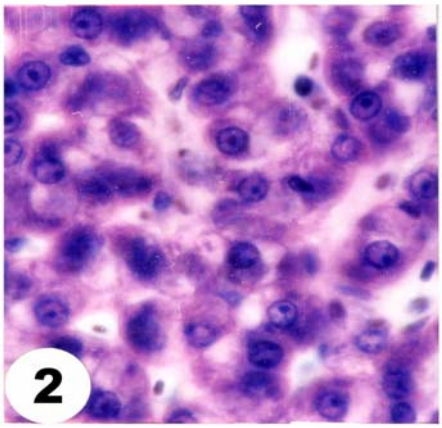

Fig 2: Magnified portion showing polygonal liver cells and blood sinusoids. H & E. X1000.

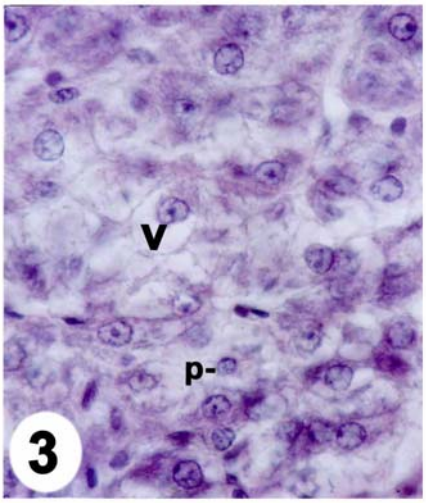

Fig 3: Photomicrograph of a section of fish liver administered garlic for 5 days, showing vacuoles (V), pycnotised nuclei (P). H & E. X1000.

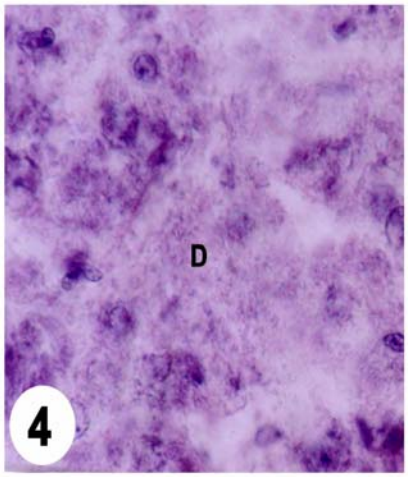

Fig 4: Photomicrograph of a section of fish liver administered garlic for 5 days, showing damaged hepatic cells (D). H & E. X1000.

Table 1: Effect of garlic (G) treatment on some serum constituents in the fish, *Chrysichthys auratus*. The standard dose was 2 g/kg body weight, given either singly, or repeatedly every 48 hrs for 5 or 11 days. Data are presented as means ± SE (n=6). * = significant ($p<0.05$), **= highly significant ($p<0.01$). TFAA= Total free amino acids; AST = glutamate oxaloacetate transaminase, ALT = glutamate pyruvate transaminase, ALP = alkaline phosphatase (all in units per litre)

	Single dose.		Repeated doses			
	24h		5 days		11 days	
	Control	G	Control	G	Control	G
Glucose (mg/dL)	95.9±5.6	50.8*±6.5	97.2±7.5	44.6**±5.3	92.5±6.1	50.2**±6.6
TFAA (mg/L)	98.5±5.4	102.1±7.1	106.4±10.0	89.7±8.1	110.5±9.0	67.7*±5.5
Total lipids (g/L)	2.9±0.22	2.4±0.20	3.2±0.21	2.3*±0.23	3.4±0.35	2.1*±0.20
Triglycerides (g/L)	2.2±0.25	1.9±0.23	2.2±0.16	1.5*±0.11	2.7±0.28	1.4**±0.16
Cholesterol (mg/dL)	60.3±9.7	52.5±8.2	70.0±6.3	44.0**±4.5	66.3±6.7	40.2*±6.2
Total lipids/ triglycerides ratio %	65.8±7.9	88.3±4.6	70.2±6.2	67.5±8.7	76.3±7.6	63.6±5.5
Total protein (g/dL)	5.5±0.35	5.3±0.20	5.1±0.31	6.0±0.41	5.2±0.36	5.7±0.34
Urea (mg/L)	53.8±4.7	49.6±4.3	52.5±3.7	46.6±5.2	57.3±5.4	33.6**±2.50
AST (U/L)	34.0±2.9	31.3±3.1	37.3±3.5	35.8±5.4	35.5±3.7	36.5±4.3

ALT (U/L)	17.2±2.3	16.8±2.8	16.0±2.7	23.3±2.7	18.3±2.9	25.8±3.6
ALP (U/L)	133 .3± 15.4	122.5 ± 13.9	143.5±18.3	146.1±10.8	150.1±16.2	85.6*±11.2

Table 2: Effect of garlic (G) treatment on some liver constituents in the fish, *Chrysichthys auratus*. Abbreviations as in Table 1

	Single dose		Repeated doses			
	24h		5 days		11 days	
	Control	G	Control	G	Control	G
Glycogen (mg/g dry wt)	123±8.2	90*±6.4	135±11.2	101*±6.7	124±12.1	109±7.2
Total lipids (mg/g dry wt)	73.6±6.2	71.0±5.2	68.6±4.2	70.9±7.6	62.2±4.0	46.7*±3.7
Triglycerides	29.6±2.4	22.2±1.6	28.2±1.7	19.8±1.9	21.7±2.2	237±2.6
Total lipid/triglyceride ratio (%)	41.5±5.0	31.1±1.9	41.5±3.3	30.0*±2.9	34.3±3.0	46.6±9.0
TFAA (µg/g dry wt)	490±49.5	583±47.0	396±45.3	605**±66.0	436±52.7	450±41.7

Table 3: Effect of garlic (G) treatment on some white muscle constituents in the fish, *Chrysichthys auratus*. Abbreviations as in Table 1.

	Single dose		Repeated doses			
	24h		5 days		11 days	
	Control	G	Control	G	Control	G
Glycogen (mg/g dry wt)	12.1±1.3	14.5±1.5	10.2±0.7	15.0*±1.5	9.7±1.1	13.2*±0.8
Total lipids (mg/g dry wt)	106.6±9.9	116.6±9.6	104.7±10.3	136.7±10.7	99.8±9.1	136.7*±9.6
Triglyceride (mg/g dry wt)	38.8±3.1	41.6±4.1	42.4±3.7	44.3±3.5	34.1±2.6	56.0**±4.5
Total lipid/triglyceride ratio (%)	37.8±5.7	37.3±34.6	41.8±5.8	33.0±3.8	34.6±4.1	43.1±6.4
Cholesterol (mg/g dry wt)	4.13±0.24	3.53±0.30	4.15±0.23	3.68±0.26	3.93±0.38	3.38±0.35
TFAA µg /g dry wt	447±22	468±37	523±40	434±42	422±46	397±43

The livers of fish administered garlic for 11 days show dilatation of some blood vessels (Fig. 5). Some nuclei of the hepatocytes have the normal size but other nuclei are pycnotic. Other pathological changes observed in this group are cytoplasmic vacuolation and certain areas of hepatocytes exhibit degeneration (Fig. 6). With Masson's trichrome stain, the amount of collagenous fibres is decreased in the experimental groups relative to normal (Figs. 7, 8).

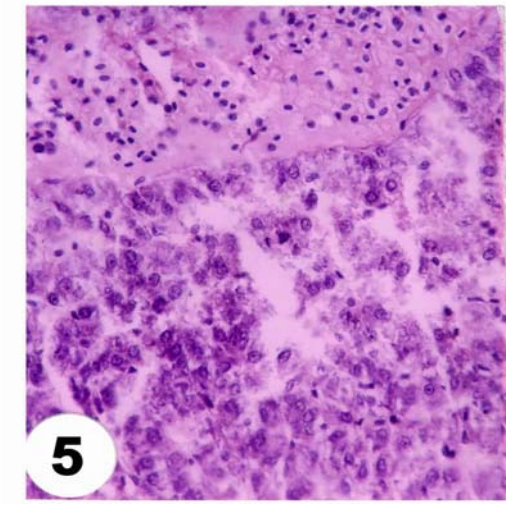

Fig. 5: Photomicrograph of a section of fish liver administered garlic for 11 days, showing dilated and congested blood vessels. H & E. X400.

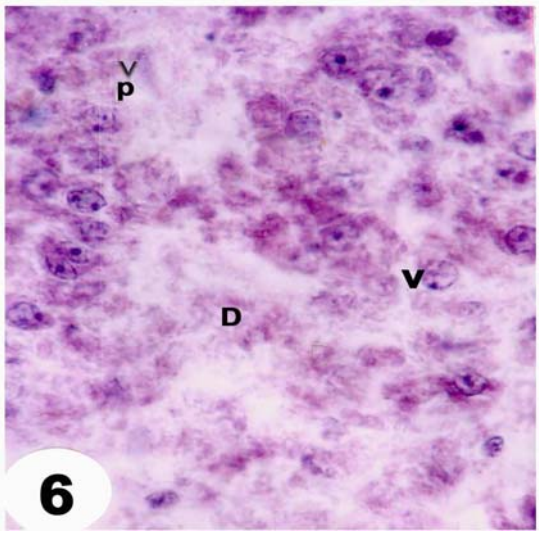

Fig. 6: Photomicrograph of a section of fish liver administered garlic for 11 days, showing pycnotised nuclei (P) , vacuoles (V) and degenerated hepatic cells (D). H & E. X1000.

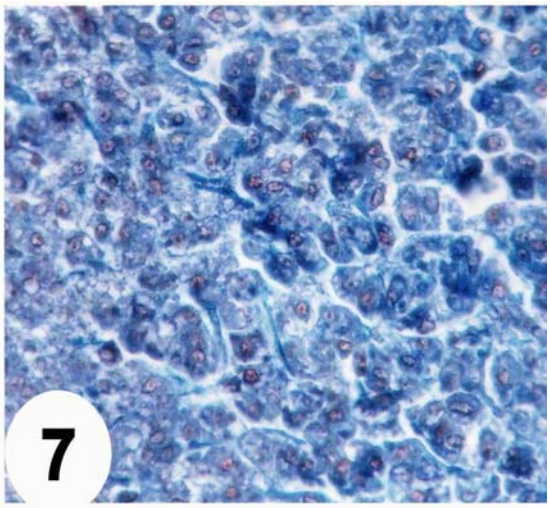

Fig. 7: Photomicrograph of a section of control fish liver showing great amount of collagenous fibres. Masson's trichrome. X400.

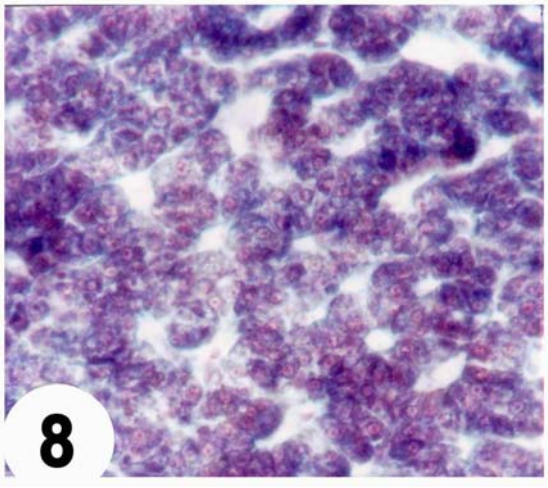

Fig. 8: Photomicrograph of a section of fish liver administered with garlic for 5 days, showing that the amount of collagenous fibres is decreased. Masson's trichrome. X400.

Using the PAS reaction, the hepatocytes of control livers show a strong positive PAS reaction (Fig. 9). There was a marked decrease in the glycogen content of hepatocytes in the experimental groups, and the cytoplasm of the liver cells was only faintly stained by the PAS reaction (Fig. 10).

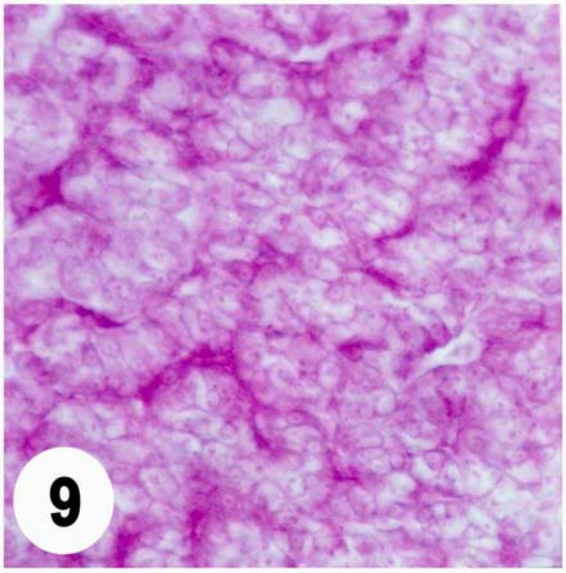

Fig. 9: Photomicrograph of a section of control fish liver showing great amount of glycogen in the hepatocytes. PAS. X400.

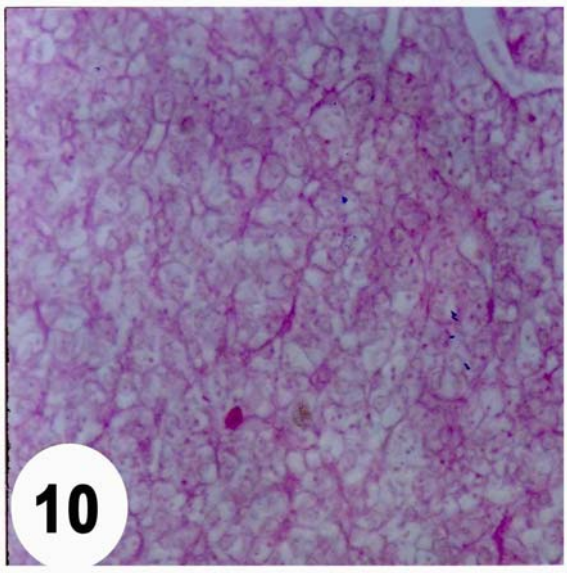

Fig. 10: Photomicrograph of a section of fish liver administered garlic for 11 days showing a remarkable reduction in glycogen. PAS. X400.

The normal kidney consists of nephrons. Each nephron is composed of a Malpighian corpuscle, and the renal and collecting tubules, as well as nests of haemopoietic tissue in between these structures. The wall of each tubule consists of a single layer of epithelial cells that rests on a basement membrane (Figs. 11, 12).

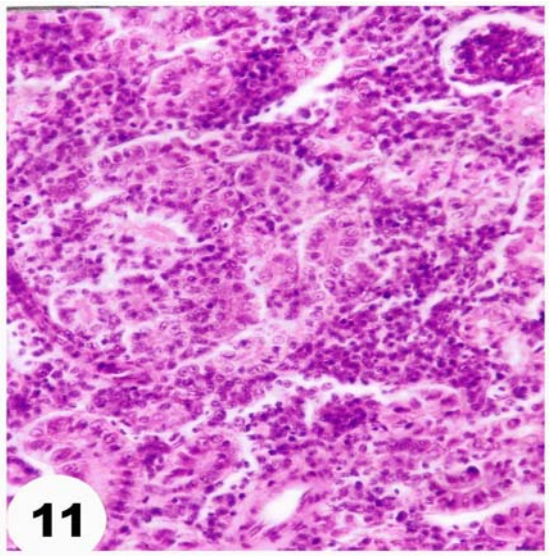

Fig. 11: Photomicrograph of a section of control fish kidney, showing the normal structure of the kidney. H & E. X400.

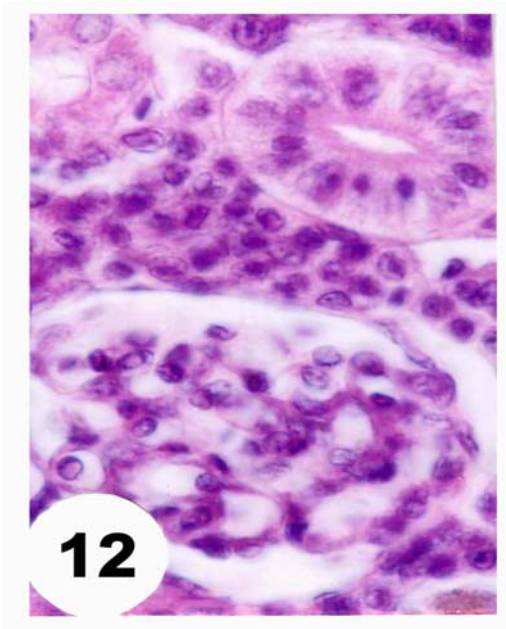

Fig. 12: Photomicrograph of a section of control fish kidney, showing Malpighian corpuscle and renal tubules. H & E. X1000.

In fish administered garlic for 5 days, the kidney shows histological alterations in its cellular constituents. The epithelial cells of some of the tubules show vacuolation and deeply stained nuclei (Fig. 13). The parietal cells of Malpighian corpuscles are also degenerated if compared with the control (Fig. 14). In fish administered garlic for 11 days, the effect of garlic becomes more conspicuous in that there is damage to some tubules, and others show necrotic cells (Fig. 15). Using Masson's trichrome stain, there is also a reduction in the amount of collagenous fibres compared to normal (Figs. 16, 17).

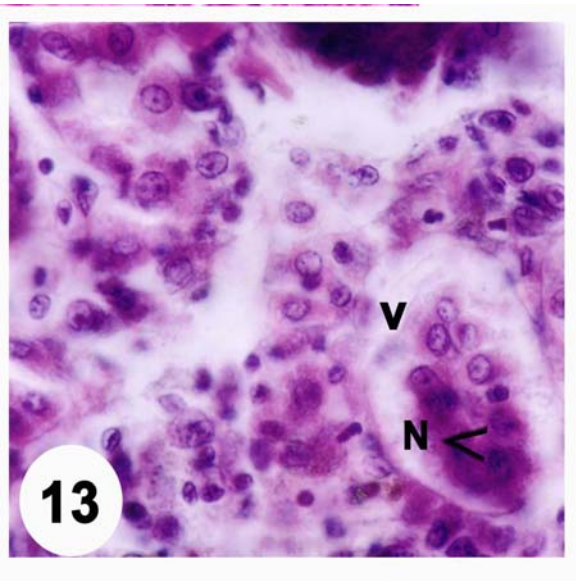

Fig. 13: Photomicrograph of a section of fish kidney administered garlic for 5 days, showing vacuolation (V) and deeply stained nuclei (N) of some renal tubules. H & E. X1000.

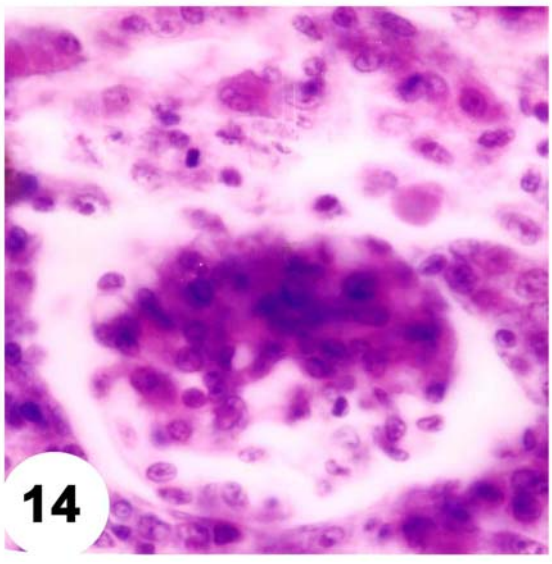

Fig. 14: Photomicrograph of a section of fish kidney administered garlic for 5 days showing degeneration of the parietal cells of Malpighian corpuscles. H & E. X1000.

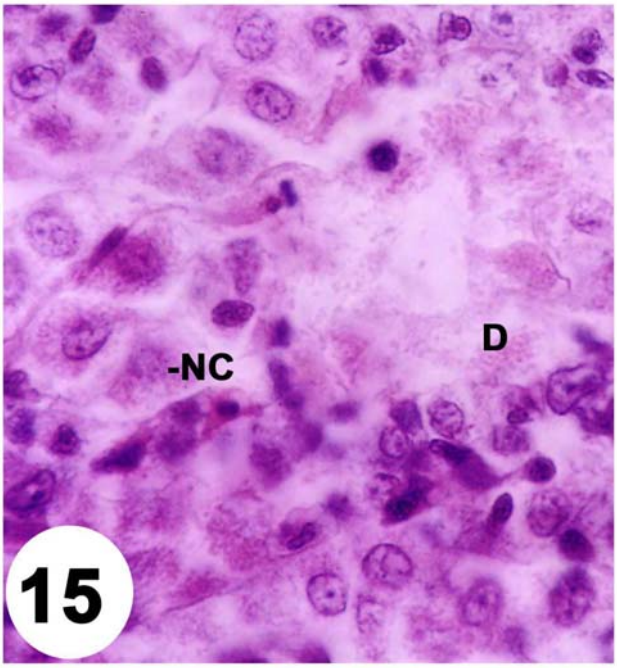

Fig. 15: Photomicrograph of a section of fish kidney administered garlic for 11days showing damage of the tubules (D) and necrotic cells (NC). H & E. X1000.

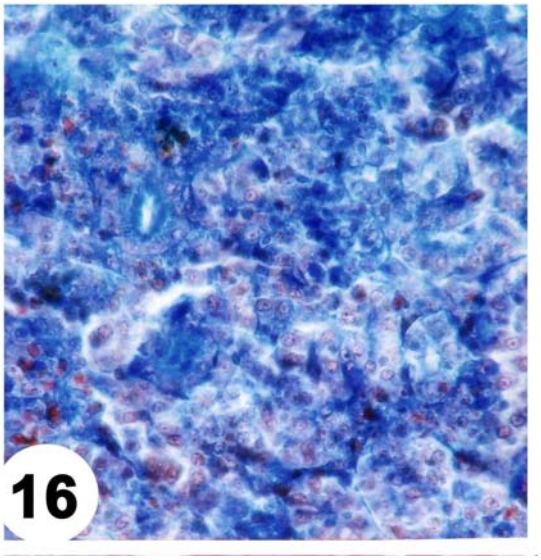

Fig. 16: Photomicrograph of a section of control fish kidney, showing high content of collagenous fibres. Masson's trichrome. X400.

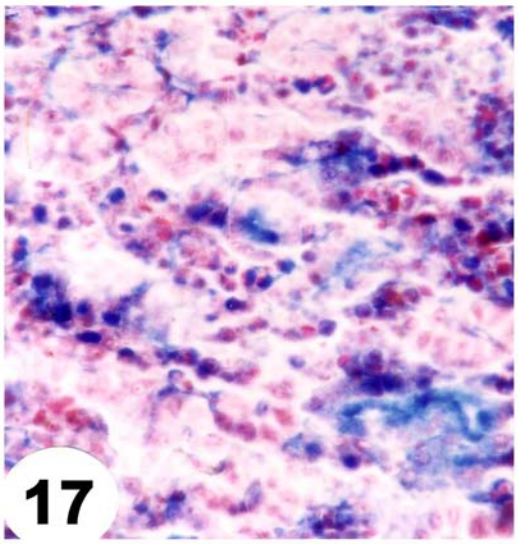

Fig. 17: Photomicrograph of a section of fish kidney administered garlic for 11 days showing that the amount of collagenous fibers is decreased. Masson's trichrome. X400.

Examination of PAS-stained sections of control kidney shows high positive reactions. These materials are highly localized in the brush borders of the tubules as well as the basement membrane of the epithelial cells of the tubules. This denotes the presence of polysaccharides (Fig. 18). After 11 days of administration of garlic, the PAS-positive material diminished in the brush borders of the tubules, indicating decrease in carbohydrate content (Fig. 19).

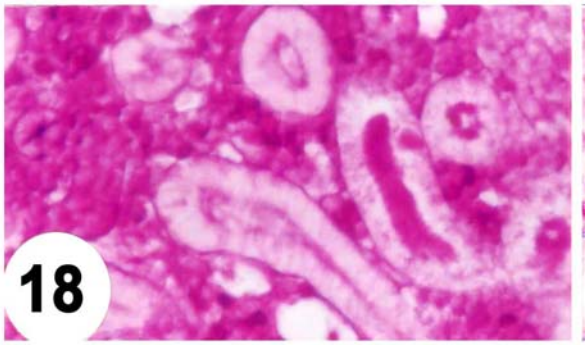

Fig. 18: Photomicrograph of a section of control fish kidney showing intense PAS positive reaction in brush borders of the tubules as well as basement membranes of the epithelial cells. PAS. X400.

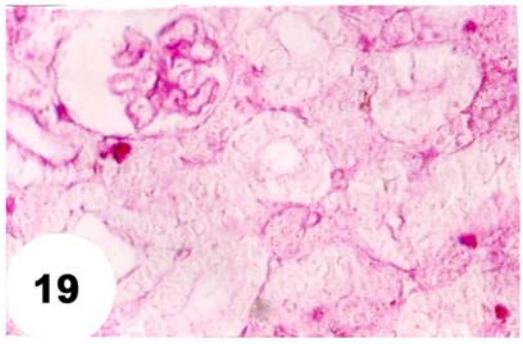

Fig. 19: Photomicrograph of a section of fish kidney administered garlic for 11 days showing decrease in PAS positive material in brush borders of the tubules. PAS. X400.

DISCUSSION

The present study showed that in all treatments the hypoglycaemic effect of garlic juice was accompanied with hepatic glycogen depletion (after 24h and 5 days) and elevated white muscle glycogen (after 5 and 11 days). These results are similar to those reported in mammals (Chang & Johnson 1980; Augusti & Sheela 1996; Preuss *et al*. 2001), where there is an association between garlic administration and the rise in circulating insulin. On studying glucose tolerance in rabbits, Jain *et al*. (1973) found that garlic juice (25g/rabbit) ameliorated hyperglycaemia as compared with an insulin-secretagogue drug (tolbutamide). In mammals, a garlic diet possesses an antidiabetic effect (Sheela & Augusti 1992, Augusti 1996), and can suppress the rise in hyperglycaemia and hyperlipidaemia in diabetic animals. The hypoglycemic effect of garlic has been recorded in various mammal species (Chang & Johnson 1980; Kasuga *et al.* 1999). However, in hypertensive rats fed diets containing 1% garlic for 45 days, blood sugar did not exhibit any significant change in spite of the elevation of circulating insulin (Preuss *et al.* 2001). It seems that 3 and 5 repeated doses of garlic were more effective in *Chrysichthys auratus*, possibly due to the high stimulatory role of garlic as an insulin secretagogue. On the other hand, Sheela & Augusti (1992) showed that a garlic derivative (200 mg/kg body wt of S-allyl cysteine sulphoxide) decreased significantly liver glucose-6-phosphatase, but increased liver hexokinase activity in alloxan-diabetic rats. This finding is consistent with the hypoglycaemic effect of garlic in the present work. Liver glycogen depletion was recorded in alloxan-diabetic mice fed garlic for 7 days (Al-Salahy & Hassanien 1993). However, garlic (2% of the diet) administration for 18 days to rats fed 1% cholesterol induced only an insignificant increase in liver glycogen (Chang & Johnson 1980). Insulin injections into fish induced hypoglycaemia, accompanied by liver glycogen depletion (Ottolenghi *et al.* 1982; Carneiro & Amaral 1983; Al-Salahy *et al.* 1994). These findings are similar to those resulting from garlic treatment. Furthermore, the administration of the insulin secretagogue drug chlorpropamide in fish leads to the same effects (Al-Salahy 2003).

Histological examination of the liver showed cytoplasmic vacuolation after garlic treatment for 5 and 11 days. A similar result was recorded by El-Ganzuri (1975), Shakoori *et al.* (1988) and El-Banhawy *et al.* (2000) after treatment with insecticides and by Khidr *et al.* (2001) after treatment with herbicides. Mollendroff (1973) and El-Banhawy *et al.* (1986) considered cytoplasmic vacuolation as a cellular defence mechanism against substances injurious to cells. Garlic induced dilation and congestion in blood vessels in liver cells in the 11-day treatment. In rats, garlic administration (36 mg/kg) leads to

engorgement of hepatic central veins with aggregated deposits and haemolyzed blood (Rahmy & Hemmaid 2001).

However, among the histological symptoms observed in the liver after treatment of garlic for 5 and 11 days was the development of cellular degeneration, something also recorded (Alnaqeeb *et al*. 1996; Banerjee *et al.* 2001; Rahmy & Hemmaid 2001) in the liver of rats treated with high doses of garlic. Bhati *et al.* (1973) suggested that liver damage elevates enzymatic levels in blood under certain pathological conditions. However, the serum enzymes AST and ALT did not show any rise, probably due to the low damaging effect of the garlic doses used here. Administration of garlic caused some degeneration in the Malpighian corpuscles (5 day treatment) and in renal tubules (11-day treatment), as reported by El-Gengaihy *et al.* (1993), Banerjee *et al.* (2001) and Attia (2002). Robbins & Angell (1976) confirmed that the degeneration of the kidney was due to autolytic action of lysosomal enzymes released out of these organelles to the ground cytoplasm or due to reabsorption of excreted protein molecules, which are greatly represented in the glomerular filterate; this is due to cellular damage. However, in the present study the unchanged serum levels of AST (an enzyme specific to the liver and kidney functions) and total protein, as well as a drop in serum urea in response to garlic treatment, illustrate that the kidney was still performing its normal function.

On the other hand, we found that the amount of collagenous fibers in the liver and kidney decreased after garlic treatment for 5 and 11 days. A similar result was observed by Mirhadi *et al.* (1990) in rabbits fed garlic. These authors attributed this finding to decreases in the activity of mitochondrial monoamine oxidase in the liver and kidney of rabbits under the effect of garlic. The histochemical studies showed liver and kidney glycogen depletion in response to garlic treatment, and the biochemical analysis gave the same result in the liver. Rahmy & Hemmaid (2001) also reported this in response to treatment with garlic (36 mg/kg), injection of cobra venom, or under the effect of both treatments. Heath (1995) attributed such glycogen reduction to depressed feeding or elevated levels of the stress hormones, adrenaline and cortisol.

Little information was available regarding the effect of garlic diet on muscle glycogen. In the present study, garlic treatment resulted in significant rise in muscle glycogen, suggesting an enhancement in muscle uptake of serum glucose, probably due to the elevated insulin release.. Muscle glycogen was also increased in alloxan-diabetic mice fed garlic extract (0.3g for each animal) for 7 days (Al-Salahy & Hassanien 1993).

Orally administered garlic for 5 and 11 days resulted in significant hypolipidaemia, hypotriglyceridaemia and hypocholesterolaemia. This result is consistent with those recorded in mammals (Bordia 1981; Mader 1990), and we know that a garlic diet has an antihyperlipidaemic effect in humans and mammals (Kaul & Parasad 1990; Mader 1990; Mirhadi *et al.* 1991; Yeh & Yeh 1994; Augusti *et al.* 2001).

Quershi *et al.* (1983) found an association between hypocholesterolaemia and a decrease in hepatic 3-hydroxy-3-methyl glutaryl-COA reductase (83%), cholesterol-α hydroxyle (51%) and fatty acid synthetase (51%) in leghorn pullets fed a diet containing 3.8% garlic paste. In humans, garlic powder (60 mg/day for 3 months) reduced plasma cholesterol and triglycerides in volunteers suffering from hypercholesterolaemia and hypertriglyceridaemia (Brosche *et al.* 1990). Consequently, the lowering effect of garlic on the serum lipids may denote that *Chrysichthys,* normally suffers from hyperlipidaemia. This result may denote variable effects of garlic juice on fish species under the same condition of treatment, possibly due to different responses of β cells in secreting insulin after garlic treatment rather than the different feeding habits of fish.

The present study indicated that garlic significantly reduced the liver total lipids in spite of its unchanged level of triglycerides after the 11-day treatment. The same treatment

caused significant elevation of white muscle total lipids and triglycerides. Such a result may be due to the stimulatory role of garlic in esterification of hepatic fatty acids producing triglycerides, which leave as rapidly as they are synthesized in the liver. This concept is based on the probable stimulatory effect of garlic on insulin release in animals (Chang & Johnson 1980; Augusti & Sheela 1996). On the other hand, insulin injection in mammals increases the rate of lipogenesis from glucose and the synthesis of triglycerides in the liver, and causes their removal as rapidly as they are synthesized (Mayes 1983). Accordingly, it could suggest that garlic enhances the lipogenic process in the myotomal musculature of *Chrysichthys* with an insignificant change in cholesterol level. In birds, dietary garlic (3% in the diet) reduces breast and thigh-muscle cholesterol in chickens suffering from high cholesterol levels (Konjufca *et al.* 1997).

Our data showed insignificant changes in both serum glutamate oxaloacetate transaminase (AST) and glutamate pyruvate transaminase (AST) in spite of the degeneration of some hepatocytes seen in histological observation. Such results suggest an inhibitory effect of raw garlic juice on the activity of liver ALT (a more specific enzyme to the liver) and AST. This concept is consistent with those reported in mammals, where garlic at different doses often decreases serum AST and ALT (Unnikerishnan *et al.* 1990; Hattori *et al.* 2001). Moreover, assay of plasma ALT activity, an indicator of liver necrosis, showed that dially disulphide isolated from garlic treatment (200 mg/kg body wt), effectively protected the liver of mice against acetaminophen (Zhao & Shichi 1998). Ajoene (20-100 mg/kg body wt) derived from garlic suppressed the rise in serum ALT activity in acetaminophen-induced liver injury in mice (Hattori *et al.* 2001). However, a high dose of garlic (2 ml garlic oil/100g body wt.) led to significant rise in serum AST in rats (Joseph *et al.* 1989). Accordingly, it could be suggested that the damage effect of garlic on liver did not reach such levels as to cause elevations of serum transaminases in the present work. Banerjee *et al.* (2001) found that garlic in low doses has the potential to enhance the endogenous antioxidant status, while at higher doses the reverse of this effect is observed. Sumiyoshi *et al.* (1984) found no toxicity symptoms in rats due to dietary garlic extract at 2g/kg 5 times/week for 5 months. However, a low dose of garlic (50 mg/kg body wt daily for 14 days) reduced ALT in mice treated with a chronic lethal dose of cyclophosphamide (Unnikerishnan *et al.* 1990).

Data of the present study showed a significant drop in serum alkaline phosphatase (ALP) in response to 11-day repeated doses of garlic. Similarly, garlic oil decreased serum ALP in streptozotocin-diabetic rats (Ohaeri 2001). However, serum ALP showed a rise only in the case of extrahepatic obstruction, intrahepatic cholestasis, infiltrative liver disease and hepatitis (McIntyre & Rosalki 1991). In contrast, the repeated doses of the present work did not induce any of these effects.

The present work showed diminished level of serum total free amino acids TFAA accompanied with significant drop in liver TFAA and insignificant change in white muscle TFAA in response to the 11-day tretament. This result may result from the elevation in insulin release in response to garlic treatment in *Chrysichthys*. Our data showed that garlic did not affect the serum total protein and decreased serum urea following the 11-day treatment. This suggests that degeneration of some of the cells in the renal tubules was not great enough to leak serum protein *via* the kidney.

In conclusion, the hypoglycaemia, hypolipidaemia, hypochosterolaemia, hypotriglyceridaemia and the decrease in the serum TFAA may be correlated to the elevation of insulin release in response to garlic treatment. In addition garlic enhanced glycogenesis and lipogenesis in white muscle, the main bulk of fish myotomal musculature (Zaitsev *et al.* 1969) and hence body mass in fish. Accordingly, a garlic diet may improve carbohydrate metabolism in fish, probably leading to lower dietary protein costs. Repeated

doses of garlic caused obvious histological changes in the organs; this probably may due to the relative high dose of garlic used in this work, but there were beneficial as well as harmful effects.

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الملخص العربى

دراسات أيضية وهستولوجية على تأثير الثوم على السمكة آكلة اللحوم: الزمار

محمد بسام الصلاحى- عبدالله بكر محمود
قسم علم الحيوان- كلية العلوم- جامعة أسيوط

تم إعطاء سمكة الزمار عصارة الثوم عن طريق الفم وكانت جرعة الثوم 2جم/كجم من وزن الجسم. وأخذت عينات من الدم والكبد والعضلات البيضاء للتحليل الكيميائي بعد 24 ساعة (جرعة واحدة) وبعد: 5 و11 يوم (جرعات متكررة كل منها 2جم/كجم من وزن الجسم كل 48 ساعة بمعدل 3 جرعات و 5 جرعات على الترتيب) كما أخذت عينات من الكبد والكلية للفحص الهستولوجى فى الفترات 5 و11 يوم. كما كانت هناك مجموعة ضابطة لكل فترة من فترات التجربة. أوضحت الدراسة حدوث انخفاض فى مستوى السكر والدهون والكوليسترول والجلسريدات الثلاثية والأحماض الأمينية الكلية فى الدم وأيضا ارتفاع فى مستوى الدهون والجليكوجين فى العضلات البيضاء بعد معاملة السمك بالثوم وخاصة بعد الجرعات المتكررة. هذه النتائج ربما ترجع إلى الدور التحفيزى للثوم فى زيادة إفراز الأنسيولين فى سمكة الزمار. وفى نفس الوقت أظهر كل من الفحص الهستولوجى والكيميائى للكبد انخفاض مستوى الجليكوجين بعد المعاملة بالثوم. وقد أظهرت الدراسة الهستولوجية أن جرعة الثوم المتكررة لمدة خمسة أيام قد أدت إلى تغيرات فى الكبد تمثلت فى تكوين فجوات فى السيتوبلازم وتضخم بعض خلاياه مع حدوث تلف واضح فى بعض الخلايا الكبدية وفى مناطق أخرى أدى إلى فقدان التنظيم العام لنسيج الكبد فى السمكة . أما الجرعة المتكررة لمدة أحد عشر يوما قد تسببت في اتساع الأوعيه الدموية ,واحتقانها علاوة على بعض الخلايا المتحلله. كذلك تأثرت أنسجة الكلية بعد خمسة أيام من إعطائها الجرعات المتكررة من الثوم حيث حدث تمزق الخلايا الجدارية لكريه مليجى، أما الأنيبيبات البولية فقد ظهرت فجوات كبيرة بها ، وأما بعد أحد عشر يوما فقد كان التأثير شديدا تمثل فى حدوث تلف واضح فى بعض الأنيبيبات البولية. من جانب آخر أظهر الفحص الهستولوجى انخفاض مستوى الكريوهيدرات فى الكلية وانخفاض كمية الألياف البيضاء فى كل من الكبد والكلية نتيجة المعالجة المتكررة من الثوم. وبالرغم من حدوث تلف لبعض الخلايا فى كل من الكبد والكلية فإن مستوى انزيمات الترانس أمينيز(AST and ALT) فى الدم لم تتغير مع حدوث انخفاض فى مستوى أنزيم الفوسفاتيز القلوى فى الدم. هذه النتيجة تظهر أن وظيفة الكبد ما زالت طبيعية كذلك فان عدم انخفاض مستوى بروتينات الدم وانخفاض مستوى البوليا يظهر أن الكلية تعمل أيضا بشكل طبيعى. بشكل عام يمكن القول أن الثوم يعمل على تحسين أيض الكربوهيدرات فى الأسماك آكلة اللحوم مثل سمكة الزمار. وأن تلف بعض الخلايا فى كل من الكبد والكلية قد يرجع إلى الارتفاع النسبى فى جرعة الثوم المستخدمة فى هذه التجربة.