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Effect of Cysteine on the Toxicity of Lead in Cooked Nile Tilapia

Abdel-Fattah M. El-Sayed[1], Sabry A. M. El-Agizy[2], El-Sayed M. Abu-Tor[3], Neveen A. El-wardany[2], Mariam A. A. Abd El-kadier[2]

Oceanography Department, Faculty of Science, Alexandria University[1]
Home Economics Department, Faculty of Specific Education, Alexandria University[2]
,Food Sciences and Technology Department, Faculty of Agriculture, Alexandria University[3]

Abstract

The aim of the study was to evaluate the effect of cysteine and its plant sources (as chickpea) to reduce the levels of lead metals in cooked Nile tilapia (*Oreochromis niloticus*). Especially, the cysteine has an active hydrogen sulfide (SH) group which can chelate the lead. These lead metals cause the contamination of fish. Cysteine with lead formed stable and non-digestible compounds in the digestive system as proved in this research, therefore, easy to get rid of. The chemical composition of Nile tilapia and (chickpea flour) were performed. Also, heavy metals concentration in Nile tilapia was measured. Confirmation experiment was performed on the experimental rats. Male rats were divided into four groups and treated with different treatments of cysteine and chickpea flour for 60 days .Such three groups were biologically compared with control group fed on the basal diet. The sensory evaluation was performed Nile tilapia chickpea (as a source of cysteine) products. The chemical composition of Nile tilapia and (chickpea flour) indicated that they contain high protein levels (15.21, 15.79, respectively) and cysteine content was 1.8, and 0.59, respectively. Also, it was noted that lead (Pb) concentration in Nile tilapia was 6.5 ppm which was the highest heavy metal. The results showed significant difference in the concentration of lead metals in blood, urine and stool. There was also a significant difference in lipid profile, complete blood count (CBC), and liver and kidney functions. The sensory evaluation indicated that added chickpea flour to some food products like Nile

tilapia has high acceptability. The study recommended that the use of chickpea flour for reducing absorption of lead metals in cooked food products should be considered.

Keyword: Cysteine- toxicity - lead- Nile tilapia- chickpea flour

1.Introduction

Fish are a good source of protein of high biological value, vitamins, minerals, and long chain polyunsaturated fatty acids. Additionally, it has delicious acceptable taste **(Palaniappan and Muthulingam, 2016)**. On the other hand, fish could represent a big hazard on public health **(Küpeli *et al.*, 2014)** . They could be a source of hazard chemicals, such as heavy metals as Iron (Fe), copper (Cu), Cadmium (Cd), Mercury (Hg), and Lead (Pb) **(Ashraf *et al.*, 2012; Uysal, 2011)**. These pollutants have many negative effects on the human health, including liver disease, kidney damage, colon cancer, pulmonary diseases, fibrosis, in addition to respiratory and distress syndromes ***(*Hezbullah *et al.*, 2016*)***. There are different sources of fish contamination with heavy metals such industrial production, mining, agriculture and transportation the burning of fossil fuels, smelting of metal, municipal wastes, fertilizers, pesticides and sewage **(Qin *et al.*, 2008 ; Sardar *et al.*, 2013)**.One of the most effective compounds in the detoxification of heavy metals in the body is sulfur-containing amino acids, such as cysteine and glutathione (GSH) **(Mohamed and Huaxin, 2015)**. The detoxification activity of glutathione is attributed specifically to the presence and availability of cysteine with glycine and glutamic acid in the body **(Sharma and Dietz, 2006; Ernst *et al.*, 2008; Sobrino *et al.*, 2014)**. Cysteine is found in many food sources, such as chickpea, celery, broccoli, garlic, onion, wheat and red pepper **(Haj Slova and Ovesna, 2016)**. However, according to the author's best knowledge, the effects of cysteine on Lead detoxification in cooked Nile tilapia have not been well investigated. It is not known whether supplemental cysteine will reduce Lead levels in cooked fish or not. Therefore, the present study was conducted to investigate the effects of supplemental cysteine sources on reducing the levels of Lead in cooked Nile tilapia.

2. Materials and methods

2.1. Preparation of samples for analysis

2.1.1. Fish samples collection

Nile tilapia *(Oreochromis niloticus)* was obtained from Barasiq area, Behera Governorate, Egypt, in April 2017. The fish were transferred to the lab in icebox, for further analyses. Fish of

similar body weight was Nile tilapia. The average weight of Nile tilapia was 240-260 g. They were washed with distilled water and packed into pre-cleaned plastic bags and carried to the analytical Lab, Faculty of Specific Education, Alexandria University using clean stainless-steel instruments on the same day. Tissues from 9 fish of the same species were collected and kept frozen at -4℃ until further analysis.

2.1.2. Chickpea flour sample

Chickpea (Cicer *arietinum, L*) was obtained from Faculty of Agriculture, Cairo University**.** Chickpea seeds used in this research were obtained from the Department of Crops Science and Technology seed bank, Cairo University. It was soaked in distilled water at room temperature for 9 hours then was dry-heated at 120 °C for 15 minutes. Finally, dried chickpea was grinded until it became firm as flour.

Cysteine and all the chemicals and reagents used in this work were of analytical grade and obtained from EL- Goumhorya Company, Egypt. The study was conducted at the Laboratory of Fish Biology, Oceanography Department, Faculty of Science, The Laboratory of Nutrition, Faculty of Specific Education, Central Laboratory, and Faculty of Agriculture - Alexandria University.

2.2. Chemical composition for Nile tilapia and chickpea flour

Proximate analysis including moisture, crude fat, crude protein, and total ash were determined according to **AOAC (2000),** Carbohydrates values were derived empirically after subtracting the other components. Cysteine were measured using the amino acid analyzers (Biochrom 30) **(AOAC 2012).** Determination of lead in fish samples which were prepared as described in **(AOCA 2000)**. **AOAC (2000)** prepared samples were used for the determination of (Pb) **AOAC (2000)**. Atomic absorption spectrophotometer PERKIN-ELMER 2380 was used to detect these heavy metals according to **AOAC (2000).**

2.3. Biological Experiment

2.3.1. Feeding experiments

Feeding experiments were done in the Animal House, Department of Home Economics, Faculty of Agriculture; Alexandria University. Twenty four healthy adult male Wistar rats were three months old, weighed 190-200 g, were purchased from Faculty of Agriculture, Alexandria University, and acclimated to lab conditions for one week. The local committee approved the experimental design and the protocol conforms to the guidelines of the National Institutes of Health. Rats were fed oral on basal diet for one week and they divided to four groups including six rats in each group.

Animals were housed in stainless steel cages at a controlled temperature (22-24 ^{o}C) and relative humidity (60-70 %) with a photoperiod of 12 hour light / 12 hour dark. All animals were given ad libitum access to tap water and standard diet that meet the nutrient requirements for growing rats. Basal diet content was prepared according to **AIN, (1980)**. During the adaptation period, the rats were fed the control diet for six consecutive days before the beginning of the experiment.

Table (1): Test diets used in the biological study

Animal groups	Diet
Group 1	Basal diet (control).
Group 2	Lead - Contaminated Nile tilapia which was cooked in the oven[1].
Group 3	Lead - Contaminated Nile tilapia with cysteine which was cooked in the oven[2]
Group 4	Lead - Contaminated Nile tilapia with chickpea flour which was cooked in the oven[3]

*1- each rat ate 3g fish, 2- each rat ate 3g fish with 0.012g cysteine 3- each rat ate 3g fish with 2g chickpea flour.

2.4. Biochemical parameters estimation

Samples were collected from each rat and serum was separated from blood. Serum samples were stored at -81°C until analysis. Lead concentration was determined in urine, stool and serum by atomic absorption spectrophotometer PERKIN-ELMER 2380. Other biochemical parameters were determined in the samples total protein **(Wu, 2006),** uric acid **(Fossati *et al*., 1980),** creatinine concentration **(Burtis *et al*., 2012),** urea concentration **(Lumeij and Remple , 1991; Young, 1995)**, alanine aminotransferase (ALT)**,** aspartate transaminase (AST) **(Tietz *et al*., 1983; Gella *et al*., 1985),** and included total cholesterol (TC) **(Grundy et al., 1993)**, triglycerides (TG) **(Banchereau *et al*., 2000**), high density lipoprotein cholesterol (HDL) **,** lipid profile low density lipoprotein (LDL) and very low density lipoprotein (VLDL) **(Expert Panel on Detection, 2001)** and oxidative stress by lipid peroxidation (MDA), all being measured by routine spectrophotometric or an automatic hitachi 902 auto-analyzer. Blood hematology parameters were done. Blood samples were collected from vein plexus in dry clean tubes with EDTA (anti-coagulant). The non -coagulated blood was used to

determine hemoglobin (HB), hematocrit and white blood cell (WBCs) by using (Bayer Adevia 120 hematology analyzer).

2.5. Preparation of fish products:

Fish products were prepared according to the methods mentioned by **Saba (1991).** Products included luncheon, nuggets, casserole white sauce fish and cannelloni**.** Each fish product was prepared using chickpea flour except the control without chickpea flour. The ingredients of samples were shown table (2).

Table (2) ingredients of samples describe

		Samples							
		Luncheon		**Nuggets**		**Casserole white sauce fish**		**cannelloni**	
		Control	Chickpea flour	Control	Chickpea flour	Control	Chickpea flour	Control	Chickpea flour
Ingredients	**Fish (g)**	100	100	100	100	100	100	100	100
	Chickpea flour (g)	-	20	-	40	-	20	-	20
	wheat flour (g)	20	-	20	-	20	-	40	-
	Powder rusk (g)	-	-	20	-	-	-	-	-
	Egg (g)	100	100	50	50	-	-	50	50
	cheddar cheese (g)	50	50	-	-	20	20	20	20
	Onion (g)	-	-	-	-	50	50	-	-
	Oil (ml)	20	20	-	-	20	20	-	-
	frying oil	-	-	200	200	20	20	50	50
	Salt (g)	3	3	3	3	3	3	3	3
	Black pepper (g)	2	2	2	2	2	2	2	2
	Fish Spices (g)	3	3	3	3	3	3	3	3

2.6. Organoleptic characteristics assessment

Organoleptic properties of Nile tilapia products (luncheon, nuggets, casserole white sauce fish, cannelloni) were conducted using 9-point hedonic rating scale **(Wichchukit and O'Mahony, 2015)**. Thirty members (students and staff from Faculty of Specific Education) of the panel were selected to assess the appearance, texture, taste, odor, color and overall acceptability of each sample. The fish samples were offered and questionnaires were

handed out, and the panel members were asked to rate the samples appearance, taste, texture, color, odor and overall acceptability.

2.7. Statistical analysis

Statistical Analysis of data was carried out using SPSS 18 statistical package programs **(Kirkpatrick and Feeney, 2012)**. A one-way analysis of variance (ANOVA) was performed followed by Schefft post hoc comparisons for the source of statistically significant difference. Differences in mean values were accepted as being statistically significant ($P \leq 0.05$).

3. Results and Discussion

3.1. Proximate composition

3.1.1. Proximate composition of Nile tilapia

The chemical of the tissues of Nile tilapia used in the present study is summarized in table (3).The tissue contains 79.75% moisture. This result agreed with **Kayan *et al.*, (2015); and Liu *et al.*, (2017)** who reported that the moisture content of Nile tilapia was 72-79%. Also, it showed that the ash content was 1.53% which is accordance with that found 1.5-2 % **(Liu *et al.*, 2017).** Further, the results in table (3) showed that tissue contains 0.85% fat This finding agree with the result that obtained by **(Liu *et al.*, 2017)** who found that the fat content of Nile tilapia 0.89%. Furthermore, crude protein content was 15.21%. Comparing with the result obtained in the present study **(Obirikorang *et al.*, 2016)** found that the protein content of Nile tilapia was 16%. Further, the results in table 3 showed that tissue contains 2.65% carbohydrates.

3.1.2. Proximate composition of chickpea flour

The chemical composition of the chickpea flour used in the present study is summarized in table (3). It contains 10.45% moisture. This result agreed with **Bozdemir *et al.*, (2015); Laxmi *et al.*, (2015); and Ogamba *et al.*, (2015)** who reported that the moisture content of Chickpea flour was 11.07 %. The ash content was 4.53% as shown in table (3). This value quite close to that found 3.72% reported by **Laxmi *et al.*, (2015); and Sindhu and Sumathi, (2015)** while is higher than 2.57% who reported by **Bozdemir *et al.*, (2015)**. The fat content of Chickpea flour was 3.34% as shown in table (3). This value is lower than that obtained by **Laxmi *et al.*, (2015)**, who reported that the fat content of Chickpea flour 5.3 % . Further, the result shown in table (3) indicated that Protein content of Chickpea flour was 15.79%. The result obtained in the present study agreed with **Bozdemir *et al.*, (2015); Laxmi *et al.*, (2015); and Ogamba *et al.*, (2015)** who reported that the Protein content of Chickpea flour was 17.1% .However, this result was lower which obtained to **Bozdemir *et al.*,**

(2015); Sindhu and Sumathi, (2015),who reported that the protein content of Chickpea flour varied between 20.50-31.4%. Furthermore, carbohydrates content of Chickpea flour as shown in table (3) was 76.33% which is in accordance with the result obtained by **Jukanti *et al.*, (2012)** who reported that the carbohydrates content of Chickpea flour 76.85%.

3.1.3. Cysteine content in Nile tilapia and chickpea flour

The result indicated that cysteine content in Nile tilapia was 1.8% as shown in table (3). This result was agreed with **Mohanty *et al.*, (2014)** who reported that cysteine was 1.2%. Furthermore, the result obtained in table (3) indicated that cysteine content in chickpea flour 0.59 %. This result was agreed with **Jukanti *et al.*, (2012)**. The above result indicated that chickpea flour contained about half the amount of cysteine present in Nile tilapia. This has been proven in the experiment of the animal is that chickpea flour is necessary for support cysteine within fish to reduce the absorption of lead in the body.

Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)	Cysteine (%)
Nile tilapia	79.75± 0.12	73.72± 0.66	4.33± 0.18	1.53± 0.14	20.41± 0.35	1.80±0.04
Chickpea fiour	10.45± 0.03	15.79± 0.09	3.34±0.04	4.53± 0.05	76.33± 0.05	0.59±0.07

Table (3): Chemical composition of Nile tilapia and chickpea in (wet weight/100g) and, amino acids of Nile tilapia and chickpea flour in (dry weight/100g protein)

Statistically significant at ($p \leq 0.05$). Data was expressed by using mean ±SD

3.1.4. Concentration of heavy metals in Nile tilapia

Table (4) showed the concentration of studied heavy metals in Nile tilapia of Barasiq farm. These heavy metals were above the level of permissible limiting especially Pb that was 6.5 mg kg^{-1} much higher than the other three metals (Cd, Cu, and Zn) which were 1.5, 1.4 and 3.9, respectively. Whereas, permissible limit of Pb,

Cd, Cu, and Zn were 0.3, 1, 20 and 40, respectively. **(Indonesia National Standard SNI 7387 (2009a), Indonesian National Standard SNI 7388 (2009b); Indonesia National Agency of Drug and Food Control (BPOM) No. 03725/B/SK/VII/89 (1998); and Hutagalung and Suwirna (1987)).**

Table (4): Heavy metals concentration *(mg kg^{-1})* in Nile tilapia

Place	Nile tilapia			
	Pb	**Cd**	**Cu**	**Zn**
Pollution area	6.5±0.14	1.51±0.07	1.41±0.13	3.9±0.09
Permissible limit (mg kg^{-1})	<0.30	<1.00	< 20.0	<40.0

3.1.5. Concentration of lead in stool, urine and serum

Table (5) showed that in groups of rats fed on lead- contaminated Nile tilapia with cysteine and lead- contaminated Nile tilapia with chickpea flour a significant (P≤ 0.05) increment in concentration of lead in stool and urine was observed while concentration of lead in serum showed a significant decrement. In contrast, groups of rats fed on lead- contaminated Nile tilapia showed a significant decrement in concentration of lead in stool and urine while concentration of lead in serum induced a significant increase. It is clear from this result that the addition of cysteine and chickpea flour allow the exit of lead with urine and stool and this indicates the lack of absorption in the digestive system, which leads to reduce the proportion of accumulation in the body and various members such as liver and kidney **(Hasanuzzaman, *et al*., 2012)**.

Table (5): Concentration of lead in stool, urine and serum

Group	Control	Lead-Contaminate Nile tilapia	Lead-Contaminated Nile tilapia +cysteine	Lead-Contaminated Nile tilapia+ chickpea	(F)	(P)
Stool	٠.2±0.01^{d}	0.55±0.13^{c}	1.04±0.02^{b}	1.71±0.06^{a}	30.85	p≤ 0.01
Urine	0.01±0.01^{d}	0.44±0.01^{c}	0.75±0.01^{b}	1.45±0.01^{a}	60.71	p≤ 0.01
Serum	0.01±0.01^{c}	0.81±0.01^{a}	0.52±0.01^{b}	0.52±0.05^{b}	56.66	p≤ 0.01

n = six for each group, Values are expressed as means ± SE

Mean values within a column not sharing common superscript letters were significantly different, (p≤ 0.05).

3.2. Biochemical parameters estimation

3.2. 1. Liver and kidney functions analysis in serum of male rat fed with test diets

Tables (6, 7) showed liver functions and kidney functions in plasma aspartate transaminase (AST), alanine transaminase (ALT), uric acid, urea and creatinine. The results indicated that groups of rats fed on lead- contaminated Nile tilapia showed a significant ($P \leq 0.05$) increment in serum (AST), (ALT), uric acid, urea and creatinine. The results also showed that in comparison with the control fed on basic diet, group fed on lead- contaminated Nile tilapia, lead-contaminated Nile tilapia with cysteine, lead-contaminated Nile tilapia with chickpea flour, showed a significant ($P \leq 0.05$) decrement in all the functions of liver and kidney including (AST), (ALT), uric acid, urea and creatinine. Because of the wide spread of enzymes (AST), (ALT), urea, uric acid and creatinine in the tissues of the body with concentrations above the level in plasma blood, and therefore, their presence in the plasma represents the level of damage in the tissues. Therefore, classified as non-functional plasma enzymes and this indicates the aggravation of damage to tissue rich in these enzymes, especially liver tissue and this is hurting the affected unsaturated fatty acids caused by free radicals, which lead to the breakdown of liver cell membranes and leakage of enzymes to plasma blood **(Todorovic, *et al.*, 2005)**.Treatment with cysteine and chickpea flour are effective to reducing the damage of unsaturated fatty acids by the free radicals of the process of lipid peroxidation and the preservation of the membranes of liver cells, which helps to keep the internal cellular components and not leak out **(El-Demerdash *et al.*, 2004).**

Table (6): Analyses of Liver functions in plasma Aspartate Transaminase (AST) and Alanine Transaminase (ALT)

Groups	Control	Lead-Contaminated Nile tilapia	Lead-Contaminated Nile tilapia +cysteine	Lead-Contaminated Nile tilapia+ chickpea	(F)	(p)
Liver functions						
AST(SGOT) (Unit/L)	85.97 ± 3.17^{e}	202.01 ± 7.03^{a}	141.66 ± 15.58^{c}	94.39 ± 9.43^{de}	39.42	$p \leq 0.01$
ALT(SGPT) (Unit/L)	25.95 ± 2.49^{d}	132.20 ± 12.85^{a}	63.54 ± 14.29^{b}	61.29 ± 6.05^{bc}	28.6	$p \leq 0.01$

Values are expressed as means ± SE; n = six for each group. Mean values within a row not sharing common superscript letters were significantly different $p<0.05$.

Table (7): Kidney functions analysis of male rat fed with test diets

Group	Control	Lead-Contaminated Nile tilapia	Lead-Contaminated Nile tilapia + cysteine	Lead-Contaminated Nile tilapia + chickpea	(F)	(p)
Kidney functions						
Uric acid (mg/dl)	1.36 ± 0.12^{a}	0.73 ± 0.06^{d}	1.37 ± 0.14^{a}	1.06 ± 0.21^{ab}	4.48	$p \leq 0.01$
Urea (mg/dl)	28.58 ± 2.43^{c}	35.41 ± 2.48^{ab}	31.33 ± 1.08^{bc}	27.75 ± 0.81^{c}	2.98	0.009
Creatinine (mg/dl)	0.78 ± 0.03^{bc}	0.89 ± 0.04^{a}	$0.81 \pm .03^{ab}$	0.67 ± 0.04^{c}	4.8	$p \leq 0.01$

Values are expressed as means ± SE; n = six for each group. Mean values within a row not sharing common superscript letters are significantly different, ($p \leq 0.05$)

3.2. 2. Lipid profile in serum of male rat fed with test diets

Analysis of lipid profile including total lipid, cholesterol, triglyceride, very low-density lipoproteins (VLDL), low-density lipoprotein (LDL), high-density lipoprotein (HDL) are shown in tables (9, 10). It can be noted that groups of male rats fed on lead-contaminated Nile tilapia showed a significant ($P \leq 0.05$) increase in serum total lipid (TL), total cholesterol (TC), LDL, VLDL and triglyceride (TG) levels and a significant decrease in the serum HDL. In comparison with the control group fed on the basal diet, the group fed on lead- contaminated Nile tilapia, lead- contaminated Nile tilapia with cysteine, lead- contaminated Nile tilapia with chickpea flour showed a significant decrement in serum TL, TC, LDL, VLDL, TG while level of HDL increased significantly. lead induced rise in serum TL, TC, LDL, VLDL and TG and fall in serum HDL in group of rats fed on lead- contaminated Nile tilapia may be due to changes in gene expression of some hepatic enzyme like HMG-CoA reductase (hydroxyl-methyl-glutamyl-CoA), which in turn depresses LDL-receptor gene expression **(Kojima *et al.*, 2005)**. 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 **(Yang *et al.*, 2003).** The cholesterol in blood serum was significantly high in groups fed on lead-contaminated Nile tilapia fish as compared to control, cysteine or chickpea flour treated groups. High cholesterol level may be due to decreased activity of cytochrome P450 enzymes (**Witmer *et al.*, 1994**). Cysteine and chickpea flour can depress the hepatic activity of lipogenic, cholesterogenic enzymes such as malic enzymes, fatty

acid synthase, glucose-6-phosphate dehydrogenase **(Metwally, 2009).** Also, The rising of level in serum lipid profile may be attributed to increasing lipolysis, mediated by increasing norepinephrine release which acts through interference with the intracellular functions of Ca+2 in the cytoplasm. Moreover, once taken into the cell, heavy metals undergo reduction to involve intracellular ascorbate and glutathione **(Chundawat and Sood 2005).** As these heavy metals exert their toxic effects by producing reactive oxygen species (ROS), chickpea flour may combat this oxidative stress through modulatory effects on reactive oxygen species (ROS) **(Kent *et al*., 2003).**

Table (9): Analyses of Lipid Profile which very low-density lipoproteins (VLDL), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL)

Group	Control	Lead-Contaminated Nile tilapia	Lead-Contaminated Nile tilapia + cysteine	Lead-Contaminated Nile tilapia + chickpea	(F)	(p)
LDL (mg/dl)	13.25 ± 0.06^{b}	15.81 ± 0.02^{a}	12.62 ± 0.17^{c}	12.5 ± 0.95^{c}	39.67	p≤ 0.01
HDL (mg/dl)	28.58 ± 1.35^{ab}	17.51 ± 0.29^{c}	30.29 ± 1.23^{ab}	27.51 ± 0.71^{ab}	4.54	p≤ 0.01
VLDL (mg/dl)	15.49 ± 0.25^{d}	23.31 ± 0.27^{bc}	11.71 ± 0.43^{e}	5.79 ± 0.07^{f}	152.5	p≤ 0.01

Values are expressed as means ± SE; n = six for each group.

Mean values within a row not sharing common superscript letters were significantly different ($p \leq 0.05$)

Table (10): Analyses of lipid profile which (total lipid, cholesterol and triglyceride)

Group	Control	Lead-Contaminated Nile tilapia	Lead-Contaminated Ni tilapia +cysteine	Lead-Contaminated Nile tilapia+ chickpea	(F)	(p)
T. lipid (mg/dl)	265.7 ± 4.02^{ab}	294.16 ± 8.16^{a}	268.58 ± 4.64^{ab}	209.16 ± 15.66^{c}	4.31	p≤ 0.01
Cholesterol (mg/dl)	48.25 ± 1.96^{cd}	62.45 ± 1.28^{ca}	47.79 ± 1.26^{cd}	43.95 ± 1.24^{d}	13.61	p≤ 0.01
Triglyceride (mg/dl)	77.01 ± 1.22^{c}	116.58 ± 1.35^{b}	58.58 ± 2.18^{d}	28.95 ± 0.35^{e}	152.57	p≤ 0.01

Values are expressed as means ± SE; n = six for each group. Mean values within a row not sharing common superscript letters were significantly different ($p \leq 0.05$).

3.2. 3. Lipid peroxidation in serum of male rat fed with test diets

Table (11) indicated that a significant ($P \leq 0.05$) increase in malondialdehyde (MDA) as compared to the control group observed in groups of male rats fed on basal diet, lead-contaminated Nile tilapia with cysteine and lead- contaminated Nile tilapia with chickpea flour as compared with of male rats fed on lead-contaminated Nile tilapia. This increase is mainly due to the contamination of Nile tilapia with lead. On the other hand, the groups of male rats fed on either lead- contaminated Nile tilapia with cysteine or chickpea flour showed a significant decrease in malondialdehyde (MDA). The effects of toxicity metals on the activity of catalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD) in rat's liver. SOD is the first line of antioxidant defense, catalyzing the conversion of O_2 to the less toxic H_2O_2. With proper activity of catalase or glutathione peroxidase, H_2O_2 is neutralized with the formation of a water molecule **(Gurer *et al.*, 1999)**. However, excessive production of free radicals may inhibit the activity of enzymes to the point where the enzyme defense becomes inefficient. Furthermore, its activity in the lead (Pb) group was significantly higher than in all other groups **(Gurer *et al.*, 1999).**

3.2. 4. Albumin and total protein in serum of male rat fed with test diets

Table (11) showed that groups of male rats fed on lead-contaminated Nile tilapia led to a significant decrease in total serum protein concentration and albumin indicating different functional disorders. Because exposure to heavy metals causes damage to the liver cells due to the permeability membranes, accompanied by changes in the tissue and function of the glomeruli and urinary ulcers due to the influence of the glomerular ulcer **(Sipos *et al.*, 2003)**. This leads to the leakage of some of the amino acids that represent the building blocks of the protein **(Mannem, 2014)**. It is a failure in the ability of liver cells to form proteins. On the other hand, the groups that were fed on lead- contaminated fish with cysteine and chickpea flour showed a significant improvement in the concentration of serum proteins, compared with the control group. The interpretation of this is that the chains of free-radical interactions prevent lipid peroxidation and preserve liver cell membranes which enable them to perform their vital functions, including protein synthesis.

Table (11): Lipid peroxidation (MDA), albumin and total proteins of male rat fed with test diets

Group	Control	Lead-Contaminated Nile tilapia	Lead-Contaminated Ni tilapia +cysteine	Lead-Contaminated Nile tilapia + chickpea flour	(F)	(p)
MDA (mg/dl)	7.09 ± 0.18^{d}	10.75 ± 0.27^{a}	7.92 ± 0.21^{d}	5.87 ± 0.31^{e}	34.77	$p \leq 0.01$
Albumin (mg/dl)	1.98 ± 0.04^{b}	2.32 ± 0.17^{ab}	2.51 ± 0.11^{a}	2.41 ± 0.06^{a}	1.08	0.002
Total protein (mg/dl)	5.98 ± 0.03^{b}	4.38 ± 0.12^{d}	6.11 ± 0.14^{a}	6.02 ± 0.11^{a}	10.29	$p \leq 0.01$

Values are expressed as means ± SE; n = six for each group. Mean values within a row not sharing common superscript letters are significantly different, ($p \leq 0.05$).

3.2.5. Complete blood count of male rat fed with test diets

Tables (12) showed the analysis of white blood cells (WBCs), hemoglobin (HB) and red blood cells (RBCs) of male rats fed on the different diets. The results indicated that the groups of rats fed on lead- contaminated Nile tilapia showed a significant ($P \leq 0.05$) decrease in (WBCs), while the other groups showed a significant increase. This is perhaps due to the damage of the genetic material of the lymphocytes, because of their treatment with contaminated fish by lead. This affects the process of maturation and differentiation of these cells. In addition, it causes the physiological and chemical properties of the lymphocytes to change, resulting in increased fluidity and loss of their polarity, making them vulnerable to crash once they enter the capillaries **(Mohammadhosen *et al.*, 2003)**.On the other hand, treatment with cysteine and chickpea flour plays an important role in maintaining white blood cell membranes from breakage and breakdown. Cysteine has the ability to correlate with heavy metals because it contains the sulfur group that works on heavy metals and the formation of a complex compound to be rid of the body **(Mohammadhosen *et al.*, 2003)**.The results table (12) showed that male rats fed on lead- contaminated Nile tilapia showed a significant ($P \leq 0.05$) decrease in hemoglobin (Hb) and red blood cells (RBCs). While, other groups induced a significant was an increase in (Hb) and (RBCs). In the present study, treatment with fish contaminated by lead led to a significant decrease in the system of red blood cells in terms of the number of red bloods, concentration of hemoglobin and the size of cells, which is the cause of the occurrence of anemia. Also, the number of red blood cells it's

appeared on the affected totals decrease, because of the impact of lead on the bone marrow which is the main source of generation, causing the reduction of the number of erythroid progenitor cells of the reduction of their ability to be divided. Due to low concentration of arthrophotin which is the most important gcolumnth factor that regulates the production of red blood cells and maturation in the bone marrow **(Stec, 2003)**. In addition to lead associated with proteins and fats caused a change in their properties and increase their fragility which leads to the crash once it enters the blood vessel capillary. So, concentration hemoglobin decreased. From the other side, treatment with cysteine and chickpea flour was played an important role in maintaining red blood cell membranes from breakage and breakdown **(Sakata *et al*., 2007).**

Table (12): Complete blood count of male rat fed with test diets

Group	Control	Lead-Contaminated Nile tilapia	Lead-Contaminated Nile tilapia +cysteine	Lead-Contaminated Nile tilapia +chickpea	(F)	(p)
WBCs (count×103/ccm)	10.91 ± 0.11^{b}	6.74 ± 0.67^{d}	16.12 ± 0.85^{a}	11.41 ± 0.25^{b}	8.69	p≤ 0.01
HB (g/dl)	14.57 ± 0.24^{a}	9.05 ± 0.51^{c}	13.46 ± 0.44^{a}	12.75 ± 0.24^{ab}	4.94	p≤ 0.01
RBCs (count×10^3/ccm)	7.53 ± 0.15^{a}	3.33 ± 0.29^{d}	6.31 ± 0.27^{b}	5.95 ± 0.58^{b}	3.46	0.003

Values are expressed as means ± SE; n = six for each group. Mean values within a row not sharing common superscript letters are significantly different, ($p \leq 0.05$).

3.3. The sensory evaluation of different products of Nile tilapia containing chickpea flour

Tables (10) show the organoleptic properties of the different products from Nile tilapia containing chickpea flour. These properties include appearance, taste, texture, color, odor and overall acceptability. The products were Nile tilapia with white sauce, Nile tilapia cannelloni, Nile tilapia nuggets, Nile tilapia luncheon. It can be noted that there were some significant differences in the mean value of some organoleptic attributes including taste, texture, color, odor and overall acceptability between all products were prepared from Nile tilapia. The mean values of the quality attributes were the same between the control products as well as the products containing chickpea flour. All the products were found to be between like moderately and like extremely.

Table (13): Organoleptic properties between the different groups according to different parameters in Nile tilapia (n = 30)

		Appearance	Taste	Texture	Color	Odor	Acceptability
Nile tilapia Luncheon	Control. L	7.83 ± 1.07^a	7.70 ± 1.76^a	7.97 ± 1.52^a	8.07 ± 1.61^a	7.63 ± 1.81^a	7.97 ± 1.75^a
	Chickpea. L	7.97 ± 1.29^a	7.73 ± 1.33^a	8.07 ± 0.98^a	8.01 ± 1.17^a	7.67 ± 1.66^a	$8.21 \pm .99^a$
	F	0.09	0.01	0.09	0.03	0.01	0.41
	($p \leq 0.05$)	0.76	0.93	0.76	0.85	0.94	0.52
Nile tilapia Nuggets	Control. N	7.83 ± 1.72^a	7.67 ± 1.39^a	7.87 ± 1.63^a	7.67 ± 1.73^a	7.40 ± 1.77^a	7.77 ± 1.45^a
	Chickpea. N	7.63 ± 1.77^a	7.10 ± 1.58^a	7.43 ± 1.48^a	7.67 ± 1.67^a	7.30 ± 1.68^a	7.57 ± 1.45^a
	F	0.19	2.15	1.16	0.00	0.05	0.28
	($p \leq 0.05$)	0.65	0.14	0.28	0.91	0.82	0.59
Nile tilapia Fish with white sauce	Control. W	8.03 ± 0.28^a	7.87 ± 0.25^a	7.83 ± 0.26^a	8.17 ± 0.22^a	7.83 ± 0.22^a	8.00 ± 0.24^a
	Chickpea. W	7.90 ± 0.26^a	8.03 ± 0.19^a	7.90 ± 0.23^a	8.13 ± 0.21^a	7.50 ± 0.27^a	8.00 ± 0.21^a
	F	0.12	0.28	0.04	0.01	0.92	0.00
	($p \leq 0.05$)	0.74	0.59	0.85	0.91	0.34	0.91
Nile tilapia Canne lloni	Control. C	8.03 ± 0.17^a	7.93 ± 0.21^a	7.93 ± 0.21^a	8.13 ± 0.21^a	8.07 ± 0.19^a	8.17 ± 0.16^a
	Chickpea .C	8.27 ± 0.29^a	8.27 ± 0.21^a	8.10 ± 0.25^a	8.13 ± 0.23^a	8.03 ± 0.23^a	8.33 ± 0.17^a
	F	0.45	1.24	0.261	0.00	0.013	0.49
	($p \leq 0.05$)	0.51	0.26	0.61	0.91	0.911	0.48

Control. L= Nile tilapia luncheon control - Chickpea. L= Nile tilapia luncheon with chickpea

Control. N = Nile tilapia nuggets control - Chickpea. N= Nile tilapia nuggets with chickpea

Control. W= Nile tilapia fish with white sauce control - Chickpea. W= Nile tilapia fish with white sauce with chickpea

Control. C= Nile tilapia cannelloni control - Chickpea. C= Nile tilapia cannelloni with chickpea

Values are expressed as means ± SE; n = six for each group. Mean values within a row not sharing common superscript letters are not significantly different, ($p \leq 0.05$).

4. Conclusion

Fish is considered a good source of nutrition for most of the population. On the other hand, fish could represent a big hazard on public health. They could be a source of hazardous chemicals such as lead (Pb) This study was conducted to identify the effects of cysteine on the toxicity of lead in cooked Nile tilapia to show the importance of adding chickpea (as a source of cysteine) to some Nile tilapia products. The study showed that the cysteine contains in chickpea flour got rid of lead in the urine and stool rats. Also, the

sensory evaluation of some Nile tilapia products which were performed by Nile tilapia with chickpea flour (such as nuggets, cannelloni, Nile tilapia with white sauce, and luncheon) showed not significant with control Nile tilapia products. Furthermore, Nile tilapia with chickpea flour products had a high general acceptance. Therefore, the study recommended that the use of chickpea flour for reducing the absorption of lead in cooked fish should be considered.

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تاثير السيستئين على سمية الرصاص فى سمك البلطى المطهى

عبد الفتاح محمد السيد[١] - صبري أحمد المرسى العجيزي[٢] -السيد محمد أبو طور[٣] – نيفين احمد الورداني[٢] - مريم أحمد علي عبد القادر[٢]

قسم علوم البحار- كلية العلوم - جامعة الإسكندرية[١]، قسم الاقتصاد المنزلي - كلية التربية النوعية – جامعة الإسكندرية[٢]، قسم علوم وتكنولوجيا الأغذية -كلية الزراعة – جامعة الإسكندرية[٣]

المستخلص العربي

تهدف الدراسة الى تقييم تأثير السيستئين وأحد مصادره النباتية (الحمص) للإقلال من مستوي الرصاص كمعدن ثقيل في أسماك البلطى المطهية. تم اجراء التحليل الكيميائى لدقيق الحمص وسمك البلطى المستخدمين فى الدراسة. وقد وجد ان نسبة البروتين فى سمك البلطى ودقيق الحمص كانت مرتفعة. وتم قياس نسبة الرصاص فى سمك البلطى ووجد أن نسبة الرصاص كانت مرتفعة فى السمك. تم إجراء تجربة على فئران التجارب ، حيث تم تقسيمهم إلى اربعة مجموعات تغذت على السمك البلطى الملوث بالرصاص ، السمك البلطى الملوث بالرصاص مع السيستئين ، السمك الملوث بالرصاص مع دقيق الحمص، بالإضافة إلى المجموعة الضابطة التى لم يتم معاملتها بأى معاملة . وجدت اختلافات معنوية بين مجموعات الفئران من خلال قياس تركيز الرصاص فى دم ، بول، وبراز الفئران المعاملة فى التجربة. كما وجدت اختلافات معنوية فى تحليل دم الفئران من حيث الدهون الكلية ، مستوى انزيمات الكبد والكلى ، الإجهاد التأكسدى ، اليوريا ، الكرياتينين، الهيموجلوبين، وخلايا الدم البيضاء. كما أظهرت نتائج التقييم الحسى أن التقبل العام كان مرتفعا عند إضافة دقيق الحمص إلى بعض المنتجات الغذائية المصنعة من سمك البلطى ودقيق الحمص وذلك مقارنة بنفس المنتجات دون إضافة دقيق الحمص. لذلك توصى الدراسة باستخدام دقيق الحمص كمصدر للسيستئين لخفض الرصاص والحد من آثاره فى سمك البلطى المطهى . كما توصى الدراسة باستخدام دقيق الحمص كمصدر للسيستئين بالنسبة المستخدمة فى هذه الدراسة فى مصانع الأغذية لتصنيع منتجات سمكية غذائية.

الكلمات المفتاحية : الرصاص – السمية – سمك البلطي – السيستئين – دقيق الحمص.