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Evaluation of the protective role of cysteine against cadmium toxicity in cooked catfish on experimental rats

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Abstract :

The study aimed to evaluate cysteine's effectiveness, derived from chickpeas, in reducing cadmium levels in cooked catfish. Especially, the active sulfhydryl (SH) group in cysteine has the ability to chelate cadmium. The catfish are contaminated because of these high cadmium levels. According to this study, cysteine and cadmium combined to generate stable, indigestible molecules in the digestive tract that are simple to eliminate through stool. The chemical composition of chickpea flour and catfish was examined. Additionally, the concentration of heavy metals in catfish was determined. The experimental rats were utilized in a confirmation experiment. Four groups of male rats were given various amounts of cysteine and chickpea flour for 60 days. The three groups were compared biologically to a control group that was fed a basal diet. Chemical analyses of catfish and chickpea flour showed high protein content, with cysteine concentrations of (1.8% and 0.59%, respectively). catfish had a cadmium concentration of 4.4 ppm. A rat confirmation experiment revealed significant differences in heavy metal concentrations, as well as a significant difference in lipid profile, complete blood count (CBC), and liver and kidney functions. The study highlights the potential of cysteine and chickpeas in alleviating cadmium contamination in fish.

تقييم الدور الوقائي للسستئين ضد سمية الكادميوم في سمك القراميط المطهي على فئران التجارب

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المسلخص:

هدفت الدراسة إلى تقييم فعالية السيستئين المشتق من الحمص في تقليل مستويات الكادميوم في سمك القراميط المطبوخ. على وجه الخصوص، تتمتع مجموعة (SH) النشطة في السيستئين بالقدرة على خلب الكادميوم. سمك القراميط ملوث بسبب مستويات الكادميوم العالية. ووفقا لهذه الدراسة، فإن السيستئين والكادميوم يتحدان لتوليد جزيئات مستقرة وغير قابلة للهضم في الجهاز الهضمي والتي يسهل التخلص منها عن طريق البراز. تم فحص التركيب الكيميائي لدقيق الحمص وسمك القراميط. بالإضافة إلى ذلك، تم تحديد تركيز المعادن الثقيلة في سمك القراميط. تم استخدام فئران التجارب. تم إعطاء أربع مجموعات من ذكور الجرذان كميات مختلفة من السيستئين ودقيق الحمص لمدة 60 يومًا. ومقارنة المجموعات الثلاث بيولوجيًا بمجموعة الكنترول التي تم تغذيتها بنظام غذائي أساسي. أظهرت التحاليل الكيميائية لدقيق الحمص وسمك القراميط وجود نسبة عالية من البروتين حيث بلغ تركيز السيستئين (1.8% و0.59% على التوالي). وكان سمك القراميط يحتوي على تركيز الكادميوم (4.4 جزء في المليون). كشفت تجربة الفئران عن اختلافات كبيرة في تركيزات المعادن الثقيلة، وكذلك اختلاف كبير في صورة الدهون، وصورة الدم الكامل، ووظائف الكبد

والكلى. تسلط الدراسة الضوء على إمكانية السيستئين ودقيق الحمص في التخفيف من التلوث بالكادميوم في الأسماك.

Evaluation of the protective role of cysteine against cadmium toxicity in cooked catfish on experimental rats

Introduction

Fish is widely recognized as a valuable source of high-quality protein, essential nutrients, and beneficial long-chain polyunsaturated fatty acids. Its consumption is not only favored for its nutritional content but also its delicious taste **(Palaniappan and Muthulingam, 2016).** However, the potential health hazards associated with fish consumption cannot be overlooked, particularly concerning heavy metal contamination, including Iron (Fe), copper (Cu), cadmium (Cd), Mercury (Hg), and Lead (Pb) **(Ashraf et al., 2012; Uysal, 2011).** These pollutants have been linked to various adverse health effects in humans, such as liver disease, kidney damage, colon cancer, pulmonary diseases, fibrosis, as well as respiratory and distress syndromes **(Hezbullah et al., 2016).** The sources of heavy metal contamination in fish are diverse, stemming from industrial activities, mining operations, agricultural practices, transportation activities, fossil fuel combustion, metal smelting, municipal waste disposal, use of fertilizers and pesticides, and sewage contamination **(Qin et al., 2008; Sardar et al., 2013**). In the human body, the detoxification of heavy metals largely relies on sulfur-containing amino acids, with cysteine and glutathione (GSH) playing pivotal roles in this process **(Mohamed and Huaxin, 2015).** The detoxification efficiency of glutathione is closely tied to the availability of cysteine, alongside glycine and glutamic acid, within the body **(Sharma and Dietz, 2006; Ernst et al., 2008; Sobrino et al., 2014).** Cysteine is naturally found in various food sources, including chickpeas, celery, broccoli, garlic, onions, wheat, and red pepper **(Haj Slova and Ovesna, 2016).** Despite this knowledge, there remains a significant gap in understanding the specific impact of cysteine on cadmium detoxification in cooked catfish, and whether supplemental cysteine can effectively reduce cadmium levels in cooked fish.

Therefore, the primary objective of this study is to investigate the potential effects of supplemental cysteine sources on reducing cadmium levels in cooked catfish.

Materials and methods

Preparation of samples for analysis

Fish samples collection.

Catfish *(clarias gariepinus)* was obtained from Barasiq area, Behera Governorate, Egypt. The fish were transferred to the lab in an icebox, for further analysis. 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.

The weight of one catfish is 650 -750 grams, and all pieces were used to conduct the experiment. Tissues from three fish of the same species were collected and kept frozen at -18℃ until further analysis.

Chickpea flour sample

Chickpea (*cicer arietinum,* L) 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 nine hours then was dehydrated at 120 °c for 15 minutes. Finally, the 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.

Chemical composition for catfish and chickpea flour

Proximate analysis including moisture, crude fat, crude protein, and total ash were determined according to **AOAC (2000),** carbohydrate values were derived empirically after subtracting the other components. Cysteine was measured using the amino acid analyzers (Biochrom 30) **(AOAC 2012).** Determination of Cd, Pb, Cu and Zn in fish samples which were prepared as described in **AOAC (2000)**.

The atomic absorption spectrophotometer PERKIN-ELMER 2380 was used to detect these heavy metals according to **AOAC (2000).**

Biological Experiment

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 orally on basal diet for one week and they divided into 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 a standard diet that meets 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 biological study.

Animal groups	Diet
Group 1	Basal diet (control).
Group 2	cadmium - contaminated catfish which was cooked in the oven[1].
Group 3	cadmium - contaminated catfish with cysteine which was cooked in the oven[2]
Group 4	cadmium - contaminated catfish 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.

Biochemical parameters estimation

Samples were collected from each rat and serum was separated from blood. Serum samples were stored at-18°c until analysis. Cadmium 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).

Statistical analysis

Statistical Analysis of the 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 ($P \leq 0.05$).

Results and Discussion

Proximate composition

Proximate composition of catfish

The chemical composition of the tissues of catfish used in the present study is summarized in Table (2).The tissue contains 75.89% moisture. This result agreed with **Kayan et al., (2015); and Liu et al., (2017)** who reported that the moisture content of catfish was 72-79%. Also, it showed that the ash content was 1.02 % which is in accordance with that found 1.5-2 % **(Liu et al., 2017).** Further, the results in Table (2) showed that tissue contains 1.27% fat. This finding agrees with the result obtained by **(Liu et al., 2017)** who found that the fat content of catfish 0.89%. Furthermore, crude protein content was 17.44%. comparing with the result obtained in the present study **(Obirikorang et al., 2016)** found that the protein content of catfish was 16%. Further, the results in Table (2) showed that tissue contains 4.35% carbohydrate.

Proximate composition of chickpea flour

The chemical composition of the chickpea flour used in the present study is summarized in Table (2). It contains 10.45% moisture. This result agreed with **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 (2). This value is quite close to that found 3.72% reported by **Laxmi et al., (2015); and Sindhu and Sumathi, (2015)**. The fat content of chickpea flour was 3.34% as shown in Table (2). This value is lower than that obtained by **Laxmi et al., (2015)**, who reported that the fat content of chickpea flour was 5.3 % . Further, the result shown in Table (2) indicated that protein content of chickpea flour was 15.79%. The result obtained in the present study agreed with **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 **Sindhu and Sumathi, (2015)**,who reported that the protein content of chickpea flour varied between 20.50-31.4%. Furthermore, carbohydrate content of chickpea flour as shown in Table (2) was 76.33% which is in accordance with the result obtained by **Jukanti et al., (2012)** who reported that the carbohydrate content of chickpea flour 76.85%.

Cysteine content in catfish and chickpea flour

The result indicated that cysteine content in catfish was 1.82% as shown in Table (2). This result was agreed with **Mohanty et al., (2014)** who reported that cysteine was 1.2%. Furthermore, the result obtained in Table (2) indicated that cysteine content in chickpea flour was 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 catfish. This has been proven in the experiment of the animal that chickpea flour is necessary for supporting cysteine within fish to reduce the absorption of cadmium in the body.

Table (2): Chemical composition of catfish and chickpea (wet weight/100/ g) and cysteine of catfish and chickpea flour in (dry weight/100 /g protein)

Sample	Moisture content (%)	Crude protein	Crude fat	Ash	Carbohydrate	Cysteine g/100g sample

Catfish	75.89 ± 0.16	17.44± 0.57	1.27± 0.16	1.02 ± 0.02	4.35± 0.65	1.82± 0.16
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

Data was expressed by using mean ±SD.

Concentration of heavy metals in catfish

Table (3) shows the concentration of studied heavy metals in catfish of Barasiq farm. These heavy metals were above the level of permissible limiting especially cd that was 8.29 mg kg $^{-1}$ much higher than the other three metals (Pb, Cu, and Zn) which were 2.6, 2.07and 3.5, 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 (3): Heavy metals concentration *(mg kg^{-1})* in catfish

Place	**catfish**			
	Pb	**Cd**	**Cu**	**Zn**
Pollution area	2.6±0.11	8.29±0.09	2.07±0.18	3.5±0.14
Permissible limit (mg kg^{-1})	<0.30	<1.00	< 20.0	<40.0

Concentration of cadmium in stool, urine and serum

Table (4) showed that in groups of rats fed on cadmium - contaminated catfish with cysteine and cadmium - contaminated catfish with chickpea flour a significant (P≤ 0.05) increment in concentration of cadmium in stool and urine was observed while concentration of cadmium in serum showed a significant decrement. In contrast, groups of rats fed on cadmium - contaminated catfish showed a significant decrement in concentration of cadmium in stool and urine while concentration of cadmium in serum induced a significant increase. It is clear from this result that the addition of cysteine and chickpea flour allow the exit of cadmium 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 (4): Concentration of cadmium in stool, urine and serum

	Control	Cadmium - contaminated catfish	Cadmium - contaminated catfish +cysteine	Cadmium - contaminated catfish + chickpea	(F)	(P)
Stool	0.02±0.01^{b}	0.79±0.02^{b}	1.71±0.02^{b}	3.58±0.09^{a}	30.85	p≤ 0.01
Urine	0.01±0.01^{b}	0.58±0.01^{b}	1.12±0.01^{b}	2.73±0.01^{a}	60.71	p≤ 0.01
Serum	0.01±0.01^{a}	0.32±0.01^{a}	0.12±0.01^{a}	0.19±0.01^{b}	56.66	p≤ 0.01

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

Biochemical parameters estimation

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

Tables (5, 6) 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 cadmium - contaminated catfish showed a significant (P≤ 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 cadmium - contaminated catfish, cadmium -contaminated catfish with cysteine, cadmium - contaminated catfish with chickpea flour, showed a significant (P≤ 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 (5): Analyses of Liver functions in plasma Aspartate Transaminase (AST) and Alanine Transaminase (ALT)

Groups	Liver functions	
	AST(SDOT) (Unit /I)	ALT(SDPT) (Unit /I)
Control	85.97 ± 3.17^{b}	25.95 ± 2.49^{d}
Contaminated catfish	177.12 ± 3.45^{a}	129.93 ± 4.29^{a}
Contaminated catfish+ cysteine	82.29 ± 2.09^{bc}	40.71 ± 1.18^{c}
Contaminated catfish+ chickpea flour	84.12 ± 3.35^{b}	48.54 ± 1.76^{b}
(F)	39.42	28.6
(p)	0.001	0.001

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 (6): Kidney functions analysis of male rat fed with test diets.

Groups	Kidney functions		
	Uric acid (mg /dI)	Urea (mg /dI)	Creatinine (mg /dI)
Control	1.36 ± 0.12^{a}	28.58 ± 2.43^{b}	0.78 ± 0.03^{b}
Contaminated catfish	0.91 ± 0.09^{b}	32.33 ± 2.33^{a}	0.82 ± 0.03^{a}
Contaminated catfish+ cysteine	0.79 ± 0.04^{b}	37.08 ± 3.06^{a}	0.78 ± 0.03^{b}
Contaminated catfish+ chickpea flour	1.07 ± 0.04^{a}	28.54 ± 0.43^{b}	0.71 ± 0.01^{bc}
(F)	4.48	2.98	4.8
(p)	0.001	0.009	0.001

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

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 (7). It can be noted that groups of male rats fed on cadmium - contaminated catfish 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 cadmium - contaminated catfish, cadmium - contaminated catfish with cysteine, cadmium - contaminated catfish with chickpea flour showed a significant decrement in serum TL, TC, LDL, VLDL, TG while level of HDL increased significantly. Cadmium induced rise in serum TL, TC, LDL, VLDL and TG and fall in serum HDL in group of rats fed on cadmium - contaminated catfish 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 cadmium - contaminated catfish 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 (7): Analyses of lipid profile which very low-density lipoproteins (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL) total lipid, cholesterol, and triglyceride)

Groups	VLDL (mg /dI)	LDL (mg /dI)	HDL (mg /dI)	T.lipids (mg /dI)	Cholesterol (mg /dI)	T

Control	15.49±0.25[c]	13.25±0.06[c]	28.58±1.35[b]	265.7±4.02[c]	48.25±1.96[b]	77
Contaminated catfish	25.04±0.25[a]	15.44±0.88[a]	19.01±1.53[c]	305.37±16.29[a]	67.45±1.19[a]	12
Contaminated catfish+ cysteine	15.43±1.35[c]	13.76±0.26[b]	30.2±1.78[a]	268.79±1.78[b]	48.43±0.53[b]	7
Contaminated catfish+ chickpea	17.22±1.41[b]	13.58±0.32[bc]	29.79±1.23[b]	269.3±1.75[b]	44.08±0.83[c]	77
(F)	152.51	39.67	4.54	4.31	13.61	
(p)	0.001	0.001	0.001	0.001	0.001	

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

Lipid peroxidation in serum of male rat fed with test diets

Table (8) indicated that a significant ($P\le 0.05$) increase in malondialdehyde (MDA) as compared to the control group observed in groups of male rats fed on basal diet, cadmium -contaminated catfish with cysteine and cadmium - contaminated catfish with chickpea flour as compared with of male rats fed on cadmium - contaminated catfish. This increase is mainly due to the contamination of catfish with cadmium. On the other hand, the groups of male rats fed on either cadmium - contaminated catfish 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 cadmium group was significantly higher than in all other groups **(Gurer et al., 1999).**

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

Table (8) showed that groups of male rats fed on cadmium - contaminated catfish 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 cadmium - 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 (8): Lipid peroxidation (MDA), albumin and total proteins of male rat fed with test diets

Group	MDA (mg/dl)	Albumin (mg/dl)	Total proteins (mg/dl)
Control	7.09 ± 0.18^{b}	1.98 ± 0.04^{b}	5.98 ± 0.03^{b}
Contaminated catfish	9.97 ± 0.35^{a}	2.34 ± 0.03^{a}	4.51 ± 0.07^{c}
Contaminated catfish+ cysteine	5.87 ± 0.26^{c}	2.12 ± 0.03^{ab}	6.69 ± 0.11^{a}
Contaminated catfish+ chickpea flour	5.12 ± 0.26^{c}	2.15 ± 0.03^{ab}	6.67 ± 0.06^{a}
(F)	34.77	1.08	10.29
(p)	0.001	0.002	0.001

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

Complete blood count of male rat fed with test diets.

Tables (9) 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 cadmium - contaminated catfish 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 cadmium. 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 **(Mohammad 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 **(Mohammad et al., 2003)**.The results in Table (9) showed that male rats fed on cadmium - contaminated catfish 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 catfish 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 cadmium 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, cadmium associated with proteins and fats caused a change in their properties and increased their fragility which leads to the crash once it enters the blood vessel capillary. So, concentration hemoglobin decreased. On the other side, treatment with cysteine and chickpea flour played an important role in maintaining red blood cell membranes from breakage and breakdown **(Sakata et al., 2007).**

Table (9): Complete blood count of male rat fed with test diets

Group	**WBCs (count×10^3/ccm)**	**HB (g/dl)**	**RBCs (count×10^3/ccm)**	**HCT (%)**
Control	10.91 ± 0.11^{b}	14.57 ± 0.24^{a}	7.53 ± 0.15^{a}	48.57 ± 1.21^{a}
Contaminated catfish	10.11 ± 1.31^{bc}	9.72 ± 0.17^{b}	3.98 ± 0.17^{b}	42.27 ± 1.44^{ab}

Contaminated catfish+ cysteine	11.31 ± 1.88^{a}	13.02 ± 0.21^{a}	7.23 ± 0.17^{a}	45.88 ± 0.85^{a}
Contaminated catfish+ chickpea	7.57 ± 0.73^{c}	15.05 ± 1.99^{a}	6.71 ± 0.36^{ab}	41.06 ± 5.81^{b}
(F)	8.69	4.94	3.46	3.17
(P)	0.001	0.001	0.003	0.006

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

Conclusion

Fish is considered a good source of nutrition for most of the population. On the other hand, fish could represent a big hazard to public health. They could be a source of hazardous chemicals such as cadmium (Cd) This study was conducted to identify the effects of cysteine on the toxicity of cadmium in cooked catfish to show the importance of adding chickpea (as a source of cysteine) to some catfish products. The study showed that the cysteine contained in chickpea flour got rid of cadmium in the urine and stool rats. Therefore, the study recommended that the use of chickpea flour for reducing the absorption of cadmium in cooked fish should be considered.

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