Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/g0kmfr57

IMPACT OF CUMIN (*Cuminum cyminum*) EXTRACT ON CHROMIUM INDUCED HARMS TO GROWTH, HEMATOLOGY, BIOCHEMICAL AND HISTOLOGICAL PARAMETERS IN THAILA (*Catla catla)*

Misbah Tasleem[1], Dr Amara Akhtar[2*], Attiqa Naseem[3], Muhammad Munawar Ramzan[4], Misbah Mobeen[5], Muhammad Luqman[6]

[1, 2*, 3, 4, 6] Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan
[5] Department of Pure and Applied Biology Bahauddin Zakariya University Multan, Pakistan

***Corresponding Author**: Dr Amara Akhtar
*Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan
amara.akhtar47@gmail.com

ABSTRACT

Aquaculture is the fastest fish-growing sector that meets the rising demands of food security worldwide. Chromium toxicity from human activities exerts significant threats to aquatic life. The present study was conducted to evaluate growth performance, hematological, liver and kidney enzymes and histological harms because of chromium accumulation in Catla. It also showed the ameliorative effects of cumin against chromium toxicity. The fifty-two juveniles were split equally into four treatments. T0 was the control treatment (commercial feed). T1 was treated with chromium (8mg/L). T2 was treated with Cumin seed extract (10g/kg). T3 was co-treated with Chromium (8mg/L) and Cumin seed extract (10g/kg). Highest weight gain, absolute weight gain, Specific growth rate, feed conversion ratio and feed intake were observed in treatment T2 with mean values of 98.124±52.06, 388.06±1.022, 1290±0.843, 0.92±0.492 and 344.00±0.592 respectively while the minimum values were observed in treatment T1 with the mean values of 75.136±42.10868, 318.27±0.984, 1070+1.954, 0.060156, 1.79±0.492 and251.43±0.00365 respectively. RBCs, hemoglobin, hematocrit, MCV, MCH and MCHC were significantly increased in T2 with the mean values of 2.99+0.643, 15.80±0.843, 38.07±0.15, 119.3±0.66, 50.03±0.11 and 41.70±0.16 respectively while minimum values in T1 with the mean values of 2.57±0.01, 12.40±0.11, 31.01±0.09, 113.3±0.66, 45.95±0.11 and 38.89±0.16 respectively were recorded. Results of biochemical analysis showed that significant increment in the activity of alkaline phosphatase (ALP) 258.27±0.37, alanine transaminase (ALT) 8.03±0.11 and aspartate aminotransferase (AST) 232.43±0.33 in T1 was observed while minimum mean values alkaline phosphatase 200.71±0.33, alanine transaminase 5.67±0.12 and aspartate aminotransferase 212.01±0.52 were observed in T2. Maximum creatinine and urea with mean values of 2.02±0.01 and 49.25±0.29 respectively were recorded in T1 while minimum mean values of 0.62±0.01 and 30.78±0.03 respectively were observed in T2. More histological damages were also observed in T1 as compared to T2. However administration of Cumin significantly ($P < 0.05$) restored all the above-mentioned damages instigated by Chromium. So the findings of the current study proved that Cumin may be used as a therapeutic compound against heavy metal Chromium-induced toxicity in fish.

Key words: Labeo rohita, Growth performance, Hematology, Weight Gain, Chromium Toxicity.

INTRODUCTION

Fish is the richest source of important fatty acids particularly long-chain polyunsaturated fatty acids (LCPUFA) and micronutrients. Fish has significantly more micronutrients compared to the diets derived from terrestrial animals. Eating fish flesh promotes an individual's rapid development and growth. Certain quantities particularly fatty fish reduce the chances of stroke and coronary heart diseases (Beveridge *et al.*, 2013). Fish is the best source of nutrition because it has low-calorie content and an excellent combination of proteins, vitamins, fats, lipids and minerals (Balami *et al.*, 2019). Fish flesh contains high-quality necessary amino acids, docosahexaenoic and eicosapentaenoic omega-3 fatty acids. It also contains minerals particularly iron, zinc and vitamins which are often in highly assimilative forms (Beveridge *et al.*, 2013; Golden *et al.*, 2016; Obiero *et al.*, 2019).

Plant extracts have been linked with growth stimulants in aquatic organisms (Citarasu *et al.*, 2002). When *C. catla is* fed with diets supplemented with medicinal herbs the immunological response and growth performance are improved dramatically ($P<0.05$)(Nobakht and Mehmannavaz, 2010). It has been recognized that medicinal plants stimulate appetite by encouraging the daily consumption of feed. Fish-fed medicinal plants show high weight increase, high body weight, growth percentage, high SGR and high GCE when their FCR values were low. Medicinal herbs improve nutrition digestibility and appropriate consumption which in turn promotes protein synthesis and ultimately affects growth performance (Bhatnagar and Saluja, 2019). Adding medicinal plants to the diet greatly enhances growth performance, feed utilization and weight gain (Harikrishnan *et al.*, 2022).

Heavy metal contamination has become a major issue nowadays about the aquatic ecology (Kedia *et al.*, 2014; Al-Snafi, 2016; Sheikh Asadi *et al.*, 2018).Heavy metals have a high density much greater than water. These are the most dangerous environmental toxicants. They are extensively toxic, easily accumulated and very harmful. Rapid urbanization and widespread industries have been excreting their wastes and sewage water containing heavy metals and dangerous pollutants into water bodies without any treatment. These are contaminating water channels and making aquatic life difficult (Olubukola and Victor, 2012; Merola *et al.*, 2021; Chaudhary *et al.*, 2023). Heavy metals are exceedingly dangerous to living beings even at tiny concentrations. Once they enter their bodies they cause permanent harms (Abedi *et al.*, 2013). Waste from several industries containing Chromium enters aquatic systems and introduces Chromium toxicity (Muthukumaravel and Rajaraman, 2013).

Chromium is one of the common heavy metals. Human activities bring Chromium in different forms in the aquatic ecosystems where it poses dangerous impacts on aquatic life resulting in Chromium toxicity (Mulware, 2013). Chromium poisoning in fish can cause an imbalance between antioxidants and pro-oxidants that produce oxidative stress (Abedi *et al.*, 2013). Chromium accumulates in exposed species and becomes lethal (Liao *et al.*, 2004). Chromium is the most hazardous and typically interacts with thiol groups of proteins and inhibits metabolism (Das *et al.*, 2004; Ahmed *et al.*, 2013; He *et al.*, 2014). When Cr binds to essential thiols. It blocks important metabolic processes that result in Chromium toxicity (Delnomdedieu *et al.*, 1994; Hughes *et al.*, 2011).

Medicinal herbs are employed in aquaculture not only as chemotherapeutics but also as feed supplements (Wang *et al.*, 2015) because they contain a wide range of minerals and chemicals (Chang, 2000). Numerous biological effects of medicinal plants on growth promotion, enhancing appetite, boosting immunity, acting as an antibiotic and reducing stress in fish are studied (Citarasu, 2010; Chakraborty and Hancz, 2011). The natural flora is very beneficial in enhancing health and treating a wide range of ailments (Awad and Awaad, 2017; Mustafa *et al.*, 2017).

Cumin and its derivatives provide therapeutic benefits to fish. It has several positive qualities including hepatoprotective, immunostimulant, antibacterial, growth-promoting and antioxidant effects. Cumin or its derivatives added to fish diets improve fish blood biochemical profiles, boost immune responses, guard against pathogenic bacteria invasion and lessen fish oxidative stress against heavy metal toxicity (Dorucu *et al.*, 2009). It is antiseptic, anti-inflammatory, analgesic and cancer-preventive (Al-Snafi, 2016; Sheikh Asadi *et al.*, 2018). It also shows inhibitory effects on aldose

reductase, alpha-glucosidase and tyrosinase (Kedia *et al.*, 2014; Al-Snafi, 2016; Sheikh Asadi *et al.*, 2018).

Hematological and biochemical blood biomarkers are frequently used in the identification and diagnosis of sublethal effects metals toxicity. Blood's high sensitivity to environmental changes makes it a valuable indication of environmental toxicity marker. Chromium negatively impacts the hematological and biochemical properties of fish (Zaki *et al.*, 2008; Öner *et al.*, 2009). Exposure to Chromium results in significant increases in transaminases, acid phosphatase (ACP) and alkaline phosphatase (ALP). Reduced hematocrit and erythrocyte numbers are two red blood cell abnormalities associated with Chromium toxicity (Fırat and Kargın, 2010). Hypercholesterolemia and hypoglycemia are also linked with Chromium toxicity. Changes in blood cell indices depend on the length and intensity of exposure (Steinhagen *et al.*, 2004; Prabakaran *et al.*, 2007; Vinodhini and Narayanan, 2009; Shaheen and Akhtar, 2012).

Fish is the major fatty acids and protein source. Medicinal plants mainly cumin are linked to a variety of parameters including growth, hematology and liver enzymes (Beveridge *et al.*, 2013). Cumin ameliorates the toxicity provoked by heavy metals. Hematology studies the mitigative effects of cumin on Chromium intoxicated *Catla catla (Sheikh Asadi et al., 2018).*Cumin cures a lot of illnesses associated with metabolism (Al-Snafi, 2016). The liver is responsible for many important functions such as biotransformation, digestion, metabolism and storage. It is the main organ in charge of transforming harmful compounds into non-toxic ones that serve as helpful biomarkers for toxicity assessment. Growth performance, antioxidant activity, hepatoprotective properties and hematoprotective properties are all enhanced by cumin. Medicinal herbs are utilized in feed and have pharmacological properties (Chakraborty *et al.*, 2022).

Heavy metals are accumulated day by day in the environment by human activities. Chromium is lethal on continuous exposure. Chromium toxicity cause gills lesions, create oxidative stress, imbalance enzymes and hormones and mortality. Chromium negatively impacts the nervous system. It causes toxicity and harm even at low concentrations after prolonged exposure. To manage aquatic ecosystems fish living under pollution stress have biochemical and clinical characteristics that serve as indicators (Staniek *et al.*, 2020).

Materials and Methods

Heavy metals are excessively present in our environment and affect every single living organism all over the world. Fish are aquatic organisms that have direct exposure to heavy metals through water. The goal of the current study is to examine cumin potential therapeutic benefits against Chromium toxicity.

Animals

Fifty-two *Catla catla* juveniles of normal weight and size will be kept in glass aquaria of 70 litres capacity at the University of Agriculture, Faisalabad Department of Zoology, Wildlife and Fisheries UAF Community College PARS for twenty-eight days. Prior to the start of the trial all of the fish juveniles were acclimatized. Juveniles were housed in glass aquaria with 12 hours of light and dark cycles, regular feed intake and a temperature of 25 to 28 degrees Celsius. The protocol as specified by the University of Agriculture's ethics council Faisalabad Pakistan was followed in experimenting.

Chemical used

Cumin seed extract(10g/kg) and Chromium(Cr) (8mg/kg) a well-known heavy metal were used in the conduction of experiments.

Experimental Design

C. catla juveniles were purchased from the Government Fish biodiversity hatchery in Faisalabad. Juveniles were of normal size and weight. Four treatments were established for juveniles. T_0 was regarded as the control treatment and fed with basal commercial feed along with tap water. T_1 was

treated with Chromium (8mg/L). Cumin seed extract (10g/kg) was treated with T_2. Chromium and cumin seed extract (8mg/L and 10g/kg respectively) were co-treated to T_3. Juveniles were dissected after the experiment lasted for 28 days. Blood and organ samples were taken from each group of juveniles for additional examination.

Determination of growth performance and feed utilization

Juveniles' weekly gross weights from each experimental group were used to estimate growth performance. Analysis of feed utilization and growth performance were done in terms of feed conversion ratio (FCR), specific growth rate (SGR), weight gain percentage, absolute weight gain (WG) and survival rate (%).

Weight gain (%)

WG (%) $= \frac{Final\ weight - Initial\ weight}{Initial\ Weight} \times 100$

Absolute weight gain (AWG)

Absolute weight gain (AWG) =Final weight (g) – Initial weight(g)

Specific growth rate (SGR)

SGR $= \frac{Initial\ weight(g) - Final\ Weight(g)}{Experimental\ period(Days)} \times 100$

Survival rate (%)

Survival rate (%) $= \frac{Final\ number\ of\ juveniles}{Initial\ numberof\ juveniles} \times 100$

Feed conversion ratio (FCR)

FCR $= \frac{Total\ dry\ feed\ intake(g)}{Weight\ gain\ (g)} \times 100$

Hematology

The automated hematology analyzer was used to assist in the examination of hematological parameters.

Liver and kidney enzyme analysis

The ALT, AST and ALP values indicators of liver function was measured using an ELISA kit. ELISA kits were used to measure total bilirubin and albumin levels. Additionally renal function markers including urea and creatinine clearance will be estimated using standard diagnostic kits. Every assay was conducted under the supplier's guidelines.

Histology

The liver and kidney tissues were preserved in 10% buffered formalin. Following the typical procedure for lack of water in increasing ethanol levels, clearing in xylene and staining with eosin and hematoxylin approximately 4-5 micro-meter thick slices were prepared using a Richert microtome machine and placed on slides for histopathological investigation. Slides were analyzed using a light microscope (Nikon, Japan). ImageJ software was utilized to analyze the images.

Statistical analysis

The current study's findings were presented as Mean ± SEM and calculated using One-way ANOVA (analysis of variance) with Tukey's test. The treatment groups were scrutinized with the graph pad Prism 5 software. A significance threshold of $p<0.05$ was applied (Inkielewicz-Stepniak *et al.*, 2012)

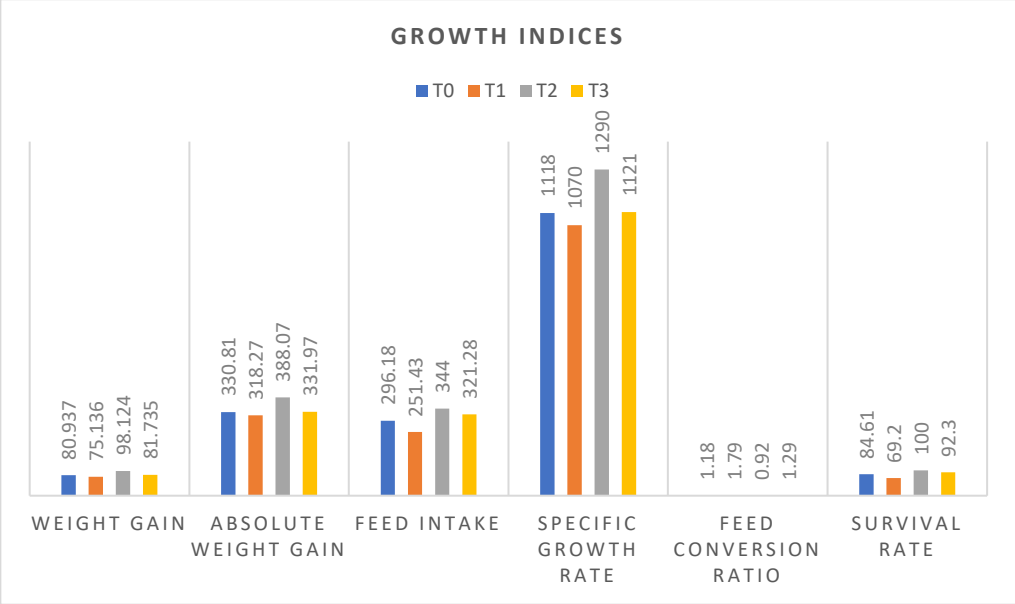
RESULTS

Growth Parameters

In the present study, different concentrations of Cumin significantly affected the growth performance and survival of *C. catla*. Considering the mean of weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR) of *C. catla*, there was a significant difference among all the treatments.

Growth Parameters	T0	T1	T2	T3
weight gain	80.937	75.136	98.124	81.735
Absolute weight gain	330.81	318.27	388.07	331.97
Feed Intake	296.18	251.43	344.00	321.28
specific growth rate	1118	1070	1290	1121
feed conversion ratio	1.18	1.79	0.92	1.29
Survival Rate	84.61	69.2	100	92.3

Table 1. Effects of Cumin extract on weight gain, absolute weight gain, feed intake, specific growth rate, feed conversion ratio and survival rate in Chromium intoxicated C. catla.



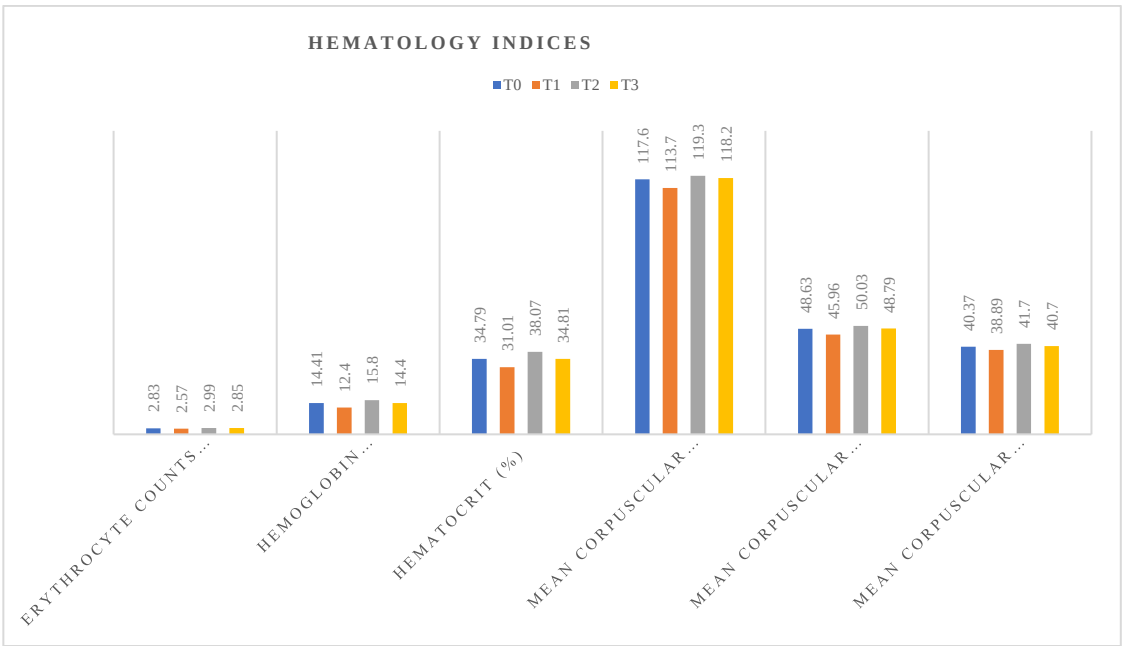
Graph 1. Graphical representation of effects of cumin extract on weight gain, absolute weight gain, feed intake, specific growth rate, feed conversion ratio and survival rate in Chromium intoxicated *C. catla*.

Hematology parameters

Following exposure of heavy metal, a significant decrease was observed in hematological profile in T_1 juveniles as compared to T_2. While a significant restoration of these parameters was noticed in T_3 as compared to T_1. Besides this, T_0 and T_3 did not express any significant difference.

Hematology Indices	T0	T1	T2	T3
erythrocyte counts (×106/µl)	2.83	2.57	2.99	2.85
Hemoglobin concentration (mg/100 mL)	14.41	12.40	15.80	14.40
Hematocrit (%)	34.79	31.01	38.07	34.81
Mean Corpuscular Volume (μm^3)	117.6	113.7	119.3	118.2
Mean Corpuscular Hemoglobin (g/dl)	48.63	45.96	50.03	48.79
Mean Corpuscular Hemoglobin concentration (g/dL)	40.37	38.89	41.70	40.70

Table 2. Effects of Cumin extract on erythrocyte counts, Hemoglobin concentration, Hematocrit, Corpuscular Volume, Mean Corpuscular Hemoglobin and Mean Corpuscular Hemoglobin concentration in Chromium intoxicated C. catla.



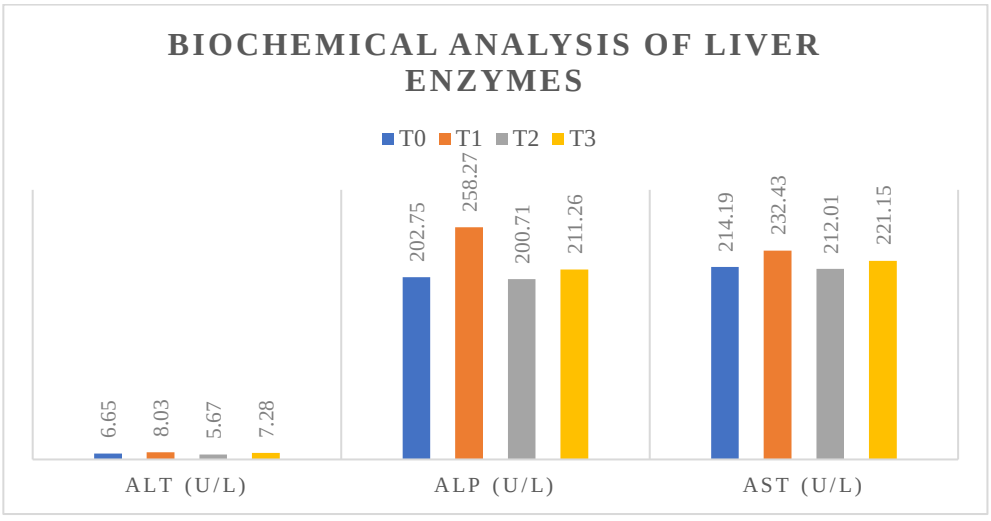
Graph 2. Graphical expression of effects of Cumin extract on erythrocyte counts, Hemoglobin concentration, Hematocrit, Corpuscular Volume, Mean Corpuscular Hemoglobin and Mean Corpuscular Hemoglobin concentration in Chromium intoxicated C. catla.

Effect of Chromium and Cumin supplementation on the liver function markers

Liver function markers were significantly increased following the exposure to heavy metals T_1 as compared to T_2. T_3 restored the level of liver function markers as compared to T_1. While no significant difference was observed between T_3 and control treatment.

Liver function marker	T0	T1	T2	T3
ALT (U/L)	6.65	8.03	5.67	7.28
ALP (U/L)	202.75	258.27	200.71	211.26
AST (U/L)	214.19	232.43	212.01	221.15

Table 3 Effect of Chromium and Cumin supplementation on the liver function markers ALT, ALP and AST.



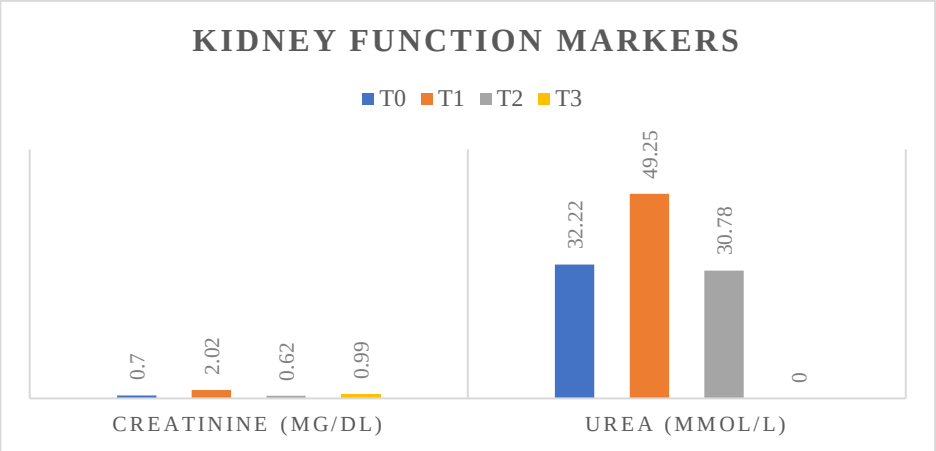
Graph 3. Effect of Chromium and Cumin supplementation on the liver function markers ALT, ALP and AST**.**

Effect of Chromium and Cumin supplementation on the kidney function markers

It was observed that T_1 significantly increased the level of creatinine and urea as compared to T_2. T_3 normalize their level as compared to T_0. While it was noticed that T_3 expressed the values similar to T_0.

Kidney Function Marker	T0	T1	T2	T3
creatinine (mg/dL)	0.70	2.02	0.62	0.99
Urea (mmol/L)	32.22	49.25	30.78	33.34n

Table 4. Effect of Chromium and Cumin supplementation on the kidney function markers creatinine and Urea



Graph 4. Graphical representation effect of Chromium and Cumin supplementation on the kidney function markers creatinine and Urea

Discussion

The aquaculture sector plays a vital role in addressing various global challenges, making it of utmost importance for both economic and nutritional reasons. Aquaculture holds a significant position as a primary source of animal protein on an international scale. Its importance stems from its widespread availability and efficient production methods, making it a key player in addressing global malnutrition and food security challenges.

The result of the present study showed that maximum weight gain was observed in T2 (98.124±0.909) which was exposed to cumin (10g/kg) and minimum weight gain observed in T1 (75.136±0.311) which was treated with 8mg/L Chromium. The results showed the significant ($P<0.05$) result in terms of weight gain, absolute weight gain and specific growth rate. This may be attributed that Chromium ingestion block digestive tracts, diminish the urge to eat and alter feeding behavior all of which reduce growth. (Wright et al., 2013). This is due to fish have redirect energy usually used for growth towards maintenance of vital functions such as the removal of heavy metals particles and their additives. Coping with other stresses such as inflammation Moos et al. (2012) and a compromised endocrine can reduce the energy available for optimal growth (Rochman et al., 2014). These results are related to the study of Cedervall et al. (2012) who reported the negative effect of Chromium on the growth of the Indian major carps. Similar results were reported by Besseling et al. (2014) showed that growth would be adversely affected by the addition of Chromium to the fish's diet. Fish growth during the acute exposure phase of the experiment showed the lowest growth in the higher Chromium treatments.

In the present study Chromium treatment (T_1) significantly decreased the level of erythrocyte count (2.57 ± 0.01), hemoglobin (12.40 ± 0.07), Hematocrit (31.01 ± 0.19), mean corpuscular hemoglobin (45.95 ± 0.27), mean corpuscular volume (113.3 ± 0.91), mean corpuscular hemoglobin concentration (42.14 ±0.14) as compared to control (T_2) erythrocyte count (2.99 ± 0.01), hemoglobin (15.80 ± 0.07), Hematocrit (38.07 ± 0.15), mean corpuscular hemoglobin (50.03 ± 0.11), mean corpuscular volume (119.3 ± 0.66), mean corpuscular hemoglobin concentration (41.70 ±0.16). It was

detected that T_2 (only *Cumin*) showed the results of hematological parameters significantly similar to that of T_0. The decrease in hematological parameters is due to Chromium exposure suppress the activity of hematopoietic stem cells which are responsible for generating various types of blood cells, including red blood cells, white blood cells, and platelets. Chromium inhibits an enzyme called δ-aminolaevulinic acid dehydratase (ALAD), which is critical for heme synthesis. As a result, the production of heme, a molecule that contains iron and is essential for hemoglobin formation in red blood cells is impaired. Insufficient heme synthesis leads to decreased hemoglobin levels and can cause anemia. The decrease in erythrocyte indices could be due to acute hemorrhages or hemolysis(Hung *et al.*, 2008), showing the rapid decrease in all hematological parameters leading to anemia. Similar findings have been reported by(Ghaffar *et al.*, 2015). Chromium exposure causes the breakdown of red blood cells, resulting in a decrease in their overall count in the bloodstream (Moradizadeh *et al.*, 2020). Chromium instigates abnormal changes in MCH, mean corpuscular hemoglobin volume (MCHV), and mean corpuscular hemoglobin concentration (MCHC), reflecting changes in the average hemoglobin content of red blood cells.

Results of present research demonstrated that T_1 (Chromium-treated group) significantly increased the level of alanine transaminase (8.03 ± 0.22), aspartate transaminase (232.43 ± 0.34) and alkaline phosphatase (258.27 ± 0.25) as compared to T_2 alanine transaminase (5.67 ± 0.12) and aspartate transaminase (212.01 ± 0.52) and alkaline phosphatase (200.71 ± 0.33 Increased levels of these enzymes is due to Chromium exposure led to the destruction of liver cell membranes and cellular structures. As a result, the content of liver enzymes normally contained within the cells is released into the bloodstream. Chromium exposure impairs mitochondrial function contributes to liver cell damage and elevates liver enzyme levels. Chromium exposure induces inflammation and cellular stress in the liver. This leads to an activation of the immune system, which further contributes to the release of liver enzymes into the bloodstream. These results were in line with previous findings in Indian carp (Vutukuru *et al.*, 2007), rats (Kalyani *et al.*, 2008), cattle (Mohanpuria *et al.*, 2008)and birds (Halder *et al.*, 2008). Increased levels of ALT and AST are indicator of As-hepatotoxicity (Roy and Bhattacharya, 2006). It has been considered that increase in ALT and AST could be due to the cellular damage or increased plasma membrane permeability, so alteration of cell metabolism due to As-intoxication could increase the enzymic activity (Ramazzotto and Carlin, 1978). The increased levels of these enzymes indicate the disordered state of hepatic tissues and subsequent hepatic dysfunction (Kandemir *et al.*, 2020).

Results of present research assessed that Chromium exposure (T_1) induced a significant increase in the level of urea (49.25 ± 0.03) and creatinine (2.02 ± 0.01) when compared to (T_2) urea (30.78 ± 0.29) and creatinine (0.69 ± 0.01). Major reason behind increase level of urea and creatinine in kidney is due to Chromium exposure decrease the rate of glomerular filtration which means fewer waste products removed from kidney. Dysfunction lead to increased excretion of essential substances and accumulation of waste product like urea and creatinine and Chromium also reduced blood flow to kidney. (Ahn *et al.*, 2017) previously described the renal toxicity induced by Chromium, highlighting the considerable reduction in kidney functions, as indicated by elevated blood urea and serum creatinine levels. various pathological lesions of As-toxicity have been reported. Increased urea level can be attributed to the kidneys failure to remove metabolic products (Haase-Fielitz *et al.*, 2009). Increased absorption of urea from renal tubules could be due to failure of the selective reabsorption property of kidney tubules. Elevated level of creatinine indicated the signs of renal failure (Padmaja *et al.*, 2009).

Histological investigation of fish kidneys subjected to Chromium treatment (T_1) revealed a significant induction of histopathological damage. This damage included tubular dilation and destruction of epithelial cells in the cortex, as well as chronic cytolysis of epithelial cells in the outer medulla, along with the presence of pyknotic nuclei. In comparison, T_0 (control group) showed minimal histopathological abnormalities. However, T_3 (Chromium +Cumin*)* exhibited a significant restoration of the histopathologic abnormalities when compared to T_1. Chromium exposure leads to the formation of lesions or abnormal growths in the kidney tissues. According to (Roy *et al.*, 2020) kidneys are

organ more vulnerable to Chromium toxicity where it induces lethal effects on renal tubular epithelium in dose dependent manner in the cockerels (Sajan *et al.*, 2022). Chromium exposure can cause histological changes of kidney, such as tubular degeneration and so on (Çimen *et al.*, 2016; Zhou *et al.*, 2017).These renal tissue damages, were consistent with previous studies conducted by (Saifi *et al.*, 2018). Upon ingestion or exposure, Chromium accumulates in renal tissues and disrupts cellular function. It disrupts cellular metabolism, impairs the function of vital enzymes, leading to oxidative stress and the generation of reactive oxygen species, causing damage to renal cells (Abdel Hamid *et al.*, 2020).

Histological investigation revealed that Chromium treatment (T_1) significantly ($P < 0.05$) induced Histopathological damage in liver of fish including hepatocytic degeneration and hepatic necrosis in the liver as compared to T_0(control group). Contrary to this T_2 exhibited significant ($P < 0.05$) restoration of the histopathologic abnormalities as compared to T_1. It was observed that T_3 results demonstrated significantly ($P < 0.05$) similar structural configuration as compared to T_0. These changes indicated the detrimental impact of Chromium on liver tissues (Santra *et al.*, 2023). Chromium-induced hepatoxicity arises from increased OS, antioxidant defense system and disturbance in mitochondrial function (Bell *et al.*, 2022). These mechanisms contribute to hepatocytic degeneration, characterized by the accumulation of lipids in the liver cells and hepatic necrosis, resulting in cell death due to severe damage (Farouk *et al.*, 2020). Cumin reverses these alterations in the liver due presence of hydroxyl group in its structural configuration which enable Cumin to reduce OS by sharing of electrons to reactive oxygen species (ROS). This sharing of electrons fulfills the need of these reactive species and normalizes into their optimum state ultimately protecting against histopathological damage caused by Chromium (Ramadan, 2020).

Cumin shows potential in mitigating the above-discussed changes induced by Chromium exposure. Cumin, a powerful antioxidant has been found to counteract the decrease in hematological parameters and liver function markers caused by Chromium. Furthermore, Cumin's protective properties have demonstrated the ability to preserve the histological integrity of the liver and kidneys, safeguarding against the detrimental effects of Chromium. The administration of Cumin exerts various health-promoting activities in aquaculture, such as improved performance, meat quality and immuno-modulation.

References

1. Abdel Hamid, E.R., N.E. Sharaf, H.H. Ahmed, A. Ahmed and A.-T.H. Mossa. 2020. In utero exposure to organochlorine pesticide residues and their potential impact on birth outcomes and fetal gender. Environmental Science and Pollution Research 27:33703-33711.
2. Abedi, Z., M.K. Khalesi and E.S. kohestan. 2013. Biochemical and hematological profiles of common carp (*Cyprinus carpio*) under sublethal effects of trivalent chromium.
3. Ahmed, M.K., M. Habibullah-Al-Mamun, E. Parvin, M.S. Akter and M.S. Khan. 2013. Chromium induced toxicity and histopathological changes in gill and liver tissue of freshwater fish, tilapia (*Oreochromis mossambicus*). Experimental and Toxicologic Pathology 65:903-909.
4. Ahn, I.E., C. Underbayev, A. Albitar, S.E. Herman, X. Tian, I. Maric, D.C. Arthur, L. Wake, S. Pittaluga and C.M. Yuan. 2017. Clonal evolution leading to ibrutinib resistance in chronic lymphocytic leukemia. Blood, The Journal of the American Society of Hematology 129:1469-1479.
5. Al-Snafi, A.E. 2016. The pharmacological activities of *Cuminum cyminum*-A review. IOSR Journal of pharmacy 6:46-65.
6. Awad, E. and A. Awaad. 2017. Role of medicinal plants on growth performance and immune status in fish. Fish and shellfish immunology 67:40-54.
7. Balami, S., A. Sharma and R. Karn. 2019. Significance of nutritional value of fish for human health. Malaysian Journal of Halal Research 2:32-34.

8. Bell, E.F., S.R. Hintz, N.I. Hansen, C.M. Bann, M.H. Wyckoff, S.B. DeMauro, M.C. Walsh, B.R. Vohr, B.J. Stoll and W.A. Carlo. 2022. Mortality, in-hospital morbidity, care practices, and 2-year outcomes for extremely preterm infants in the US, 2013-2018. Jama 327:248-263.
9. Beveridge, M.C., S. Thilsted, M. Phillips, M. Metian, M. Troell and S. Hall. 2013. Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture. Journal of fish biology 83:1067-1084.
10. Bhatnagar, A. and S. Saluja. 2019. Synergistic effects of autochthonous probiotic bacterium and Mentha piperita diets in *Catla catla* (Hamilton, 1822) for enhanced growth and immune response. Fisheries and Aquatic Sciences 22:1-14.
11. Chakraborty, R., K. Renu, M.A. Eladl, M. El-Sherbiny, D.M.A. Elsherbini, A.K. Mirza, B. Vellingiri, M. Iyer, A. Dey and A.V. Gopalakrishnan. 2022. Mechanism of chromium-induced toxicity in lungs, liver, and kidney and their ameliorative agents. Biomedicine and Pharmacotherapy 151:113119.
12. Chakraborty, S.B. and C. Hancz. 2011. Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. Reviews in Aquaculture 3:103-119.
13. Chang, J. 2000. Medicinal herbs: drugs or dietary supplements? Biochemical pharmacology 59:211-219.
14. Chaudhary, A., K. gul Javaid and E. Bughio. 2023. Toxic effects of chromium chloride on hematology and histopathology of major carp (*Labeo rohita*). The Egyptian Journal of Aquatic Research
15. Çimen, I., B. Kocatürk, S. Koyuncu, Ö. Tufanlı, U.I. Onat, A.D. Yıldırım, O. Apaydın, Ş. Demirsoy, Z.G. Aykut and U.T. Nguyen. 2016. Prevention of atherosclerosis by bioactive palmitoleate through suppression of organelle stress and inflammasome activation. Science Translational Medicine 8:358ra126-358ra126.
16. Citarasu, T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. Aquaculture International 18:403-414.
17. Citarasu, T., M.M. Babu, R. Sekar and M. Petermarian. 2002. Developing Artemia Enriched Herbal Diet for Producing Quality Larvae in Penaeiis monodo/tf Fabricius. Asian Fisheries Science 15:21-32.
18. Das, H., A.K. Mitra, P. Sengupta, A. Hossain, F. Islam and G. Rabbani. 2004. Chromium concentrations in rice, vegetables, and fish in Bangladesh: a preliminary study. Environment international 30:383-387.
19. Delnomdedieu, M., M.M. Basti, M. Styblo, J.D. Otvos and D.J. Thomas. 1994. Complexation of Chromium species in rabbit erythrocytes. Chemical research in toxicology 7:621-627.
20. Dorucu, M., U. Ispir, S. Colak, B. Altinterim and Y. Celayir. 2009. The effect of cumin seeds,cumin, on the immune response of rainbow trout, *Oncorhynchus mykiss*. Mediterranean Aquaculture Journal 2:27-33.
21. Farouk, A., S.E.-S. Saeed, S. Sharaf and M. Abd El-Hady. 2020. Photocatalytic activity and antibacterial properties of linen fabric using reduced graphene oxide/silver nanocomposite. RSC advances 10:41600-41611.
22. Ghaffar, A., R. Hussain, A. Khan, R.Z. Abbas and M. Asad. 2015. Butachlor Induced Clinico-Hematological and Cellular Changes in Fresh Water Fish *Labeo rohita* (Rohu). Pakistan veterinary journal 35
23. Golden, C.D., E.H. Allison, W.W. Cheung, M.M. Dey, B.S. Halpern, D.J. McCauley, M. Smith, B. Vaitla, D. Zeller and S.S. Myers. 2016. Nutrition: Fall in fish catch threatens human health. Nature 534:317-320.
24. Haase-Fielitz, A., R. Bellomo, P. Devarajan, D. Story, G. Matalanis, D. Dragun and M. Haase. 2009. Novel and conventional serum biomarkers predicting acute kidney injury in adult cardiac surgery—a prospective cohort study. Critical care medicine 37:553-560.

25. Halder, I., M. Shriver, M. Thomas, J.R. Fernandez and T. Frudakis. 2008. A panel of ancestry informative markers for estimating individual biogeographical ancestry and admixture from four continents: utility and applications. Human mutation 29:648-658.
26. Harikrishnan, R., G. Devi, H. Van Doan, W. Tapingkae, C. Balasundaram, J. Arockiaraj and E. Ringø. 2022. Changes in immune genes expression, immune response, digestive enzymes-antioxidant status, and growth of catla (Catla catla) fed with Astragalus polysaccharides against edwardsiellosis disease. Fish & Shellfish Immunology 121:418-436.
27. He, J., M. Wang, Y. Jiang, Q. Chen, S. Xu, Q. Xu, B.-H. Jiang and L.-Z. Liu. 2014. Chronic Chromium exposure and angiogenesis in human bronchial epithelial cells via the ROS/miR-199a-5p/HIF-1 α/COX-2 pathway. Environmental health perspectives 122:255-261.
28. Hughes, M.F., B.D. Beck, Y. Chen, A.S. Lewis and D.J. Thomas. 2011. Chromium exposure and toxicology: a historical perspective. Toxicological sciences 123:305-332.
29. Hung, R.J., J.D. McKay, V. Gaborieau, P. Boffetta, M. Hashibe, D. Zaridze, A. Mukeria, N. Szeszenia-Dabrowska, J. Lissowska and P. Rudnai. 2008. A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature 452:633-637.
30. Inkielewicz-Stepniak, I., M.W. Radomski and M. Wozniak. 2012. Fisetin prevents fluoride-and dexamethasone-induced oxidative damage in osteoblast and hippocampal cells. Food and chemical toxicology 50:583-589.
31. Kalyani, D., P.S. Patil, J.P. Jadhav and S.P. Govindwar. 2008. Biodegradation of reactive textile dye Red BLI by an isolated bacterium Pseudomonas sp. SUK1. Bioresource technology 99:4635-4641.
32. Kandemir, F.M., S. Yıldırım, S. Kucukler, C. Caglayan, E. Darendelioğlu and M.B. Dortbudak. 2020. Protective effects of morin against acrylamide-induced hepatotoxicity and nephrotoxicity: A multi-biomarker approach. Food and chemical toxicology 138:111190.
33. Kedia, A., B. Prakash, P.K. Mishra and N. Dubey. 2014. Antifungal and antiaflatoxigenic properties of *Cuminum cyminum* (L.) seed essential oil and its efficacy as a preservative in stored commodities. International journal of food microbiology 168:1-7.
34. Liao, C., J. Tsai, M. Ling, H. Liang, Y. Chou and P. Yang. 2004. Organ-specific toxicokinetics and dose–response of Chromium in tilapia *Oreochromis mossambicus*. Archives of Environmental Contamination and Toxicology 47:502-510.
35. Merola, C., A. Bisegna, G. Angelozzi, A. Conte, M.C. Abete, C. Stella, S. Pederiva, C. Faggio, N. Riganelli and M. Perugini. 2021. Study of heavy metals pollution and vitellogenin levels in brown trout (*Salmo trutta trutt*a) wild fish populations. Applied Sciences 11:4965.
36. Mohanpuria, P., N.K. Rana and S.K. Yadav. 2008. Biosynthesis of nanoparticles: technological concepts and future applications. Journal of nanoparticle research 10:507-517.
37. Moradizadeh, H., M. Heydari, R. Omidipour, A. Mezbani and B. Prévosto. 2020. Ecological effects of fire severity and time since fire on the diversity partitioning, composition and niche apportionment models of post-fire understory vegetation in semi-arid oak forests of Western Iran. Ecological Engineering 143:105694.
38. Mulware, S.J. 2013. Trace elements and carcinogenicity: a subject in review. 3 Biotech 3:85-96.
39. Mustafa, G., R. Arif, A. Atta, S. Sharif and A. Jamil. 2017. Bioactive compounds from medicinal plants and their importance in drug discovery in Pakistan. Matrix Sci. Pharma 1:17-26.
40. Muthukumaravel, K. and P. Rajaraman. 2013. A study on the toxicity of chromium on the histology of gill and liver of freshwater fish *Labeo rohita*. J. Pure Appl. Zool 1:122-126.
41. Nobakht, A .and Y. Mehmannavaz. 2010. Investigation the effects of using of *Thymus vulgaris, Lamiaceae menthapiperita, Oreganum valgare* medicinal plants on performance, egg quality, blood and immunity parameters of laying hens.
42. Obiero, K., P. Meulenbroek, S. Drexler, A. Dagne, P. Akoll, R. Odong, B. Kaunda-Arara and H. Waidbacher. 2019. The contribution of fish to food and nutrition security in Eastern Africa: Emerging trends and future outlooks. Sustainability 11:1636.

43. Olubukola, A.A. and C.A. Victor. 2012. Toxicity of industrial wastewater acting singly or in joint-ratios on *Clarias gariepinus*. American Journal of Environmental Science 8:366-375.
44. Öner, M., G. Atli and M. Canli. 2009. Effects of metal (Ag, Cd, Cr, Cu, Zn) exposures on some enzymatic and non-enzymatic indicators in the liver of *Oreochromis niloticus*. Bulletin of environmental contamination and toxicology 82:317-321.
45. Padmaja, L., C. Ravikumar, D. Sajan, I. Hubert Joe, V. Jayakumar, G. Pettit and O. Faurskov Nielsen. 2009. Density functional study on the structural conformations and intramolecular charge transfer from the vibrational spectra of the anticancer drug combretastatin-A2. Journal of Raman Spectroscopy: An International Journal for Original Work in all Aspects of Raman Spectroscopy, Including Higher Order Processes, and also Brillouin and Rayleigh Scattering 40:419-428.
46. Prabakaran, M., C. Binuramesh, D. Steinhagen and R.D. Michael. 2007. Immune response in the tilapia, Oreochromis mossambicus on exposure to tannery effluent. Ecotoxicology and environmental safety 68:372-378.
47. Ramadan, M.F. 2020. Introduction to cold pressed oils: Green technology, bioactive compounds, functionality, and applications, Cold pressed oils. Elsevier. p. 1-5.
48. Ramazzotto, L.J. and R. Carlin. 1978. Effects of DMSO on SGOT during hypothermia in adrenalectomized rats. Life sciences 22:329-336.
49. Roy, S. and S. Bhattacharya. 2006. Chromium-induced histopathology and synthesis of stress proteins in liver and kidney of *Channa punctatus.* Ecotoxicology and environmental safety 65:218-229.
50. Roy, S., S. Saxena, A. Sinha and A.K. Nandi. 2020. Dormancy/Auxin Associated Family Protein 2 of Arabidopsis thaliana is a negative regulator of local and systemic acquired resistance. Journal of plant research 133:409-417.
51. Saifi, C., A.W. Fein, A. Cazzulino, R.A. Lehman, F.M. Phillips, H.S. An and K.D. Riew. 2018. Trends in resource utilization and rate of cervical disc arthroplasty and anterior cervical discectomy and fusion throughout the United States from 2006 to 2013. The Spine Journal 18:1022-1029.
52. Sajan, B., V.N. Mishra, S. Kanga, G. Meraj, S.K. Singh and P. Kumar. 2022. Cellular automata-based artificial neural network model for assessing past, present, and future land use/land cover dynamics. Agronomy 12:2772.
53. Santra, A., M. Das, P. Datta, R. Saha, P.K. Lahiri and S. Zahir. 2023. Evaluation of Antimicrobial Effects of Three Antimicrobial Agents Against Pathogenic Root Canal Microbe Enterococcus Faecalis: An in Vitro Study. Journal of Coastal Life Medicine 11:2000-2008.
54. Shaheen, T. and T. Akhtar. 2012. Assessment of chromium toxicity in Cyprinus carpio through hematological and biochemical blood markers. Turkish Journal of Zoology 36:682-690.
55. Sheikh Asadi, M., A. Gharaei, J. Mirdar Harijani and A. Arshadi. 2018. A Comparison between dietary effects of *Cuminum cyminum* essential oil and *Cuminum cyminum* essential oil, loaded with iron nanoparticles, on growth performance, immunity and antioxidant indicators of white leg shrimp (*Litopenaeus vannamei)*. Aquaculture nutrition 24:1466-1473.
56. Staniek, H.Z., E. Król and R.W. Wójciak. 2020. The interactive effect of high doses of chromium (III) and different Iron (III) Levels on the carbohydrate status, lipid profile, and selected biochemical parameters in female wistar rats. Nutrients 12:3070.
57. Steinhagen, D., T. Helmus, S. Maurer, R.D. Michael, W. Leibold, J.P. Scharsack, A. Skouras and H.-J. Schuberth. 2004. Effect of hexavalent carcinogenic chromium on carp *Cyprinus carpio* immune cells. Diseases of aquatic organisms 62:155-161.
58. Vinodhini, R. and M. Narayanan. 2009. The impact of toxic heavy metals on the hematological parameters in common carp (*Cyprinus carpio* L.). Journal of Environmental Health Science & Engineering 6:23-28.

59. Vutukuru, S.S., N. Arun Prabhath, M. Raghavender and A. Yerramilli. 2007. Effect of Chromium and chromium on the serum amino-transferases activity in Indian major carp, *Labeo rohita*. International journal of environmental research and public health 4:224-227.
60. Wang, J.-L., X.-l. Meng, R.-h. Lu, C. Wu, Y.-T. Luo, X. Yan, X.-J. Li, X.-H. Kong and G.-X. Nie. 2015. Effects of *Rehmannia glutinosa* on growth performance, immunological parameters and disease resistance to Aeromonas hydrophila in common carp (*Cyprinus carpio* L.). Aquaculture 435:293-300.
61. Zaki, M., S. Moustafa, H. Rashad and N. Sharaf. 2008. Assessment of the hazardous effect of lead pollution on *Oreochromis niloticus*, including hematological, biochemical and immunological parameters.
62. Zhou, F., L.M. Azofra, M. Ali, M. Kar, A.N. Simonov, C. McDonnell-Worth, C. Sun, X. Zhang and D.R. MacFarlane. 2017. Electro-synthesis of ammonia from nitrogen at ambient temperature and pressure in ionic liquids. Energy & Environmental Science 10:2516-2520.