Protective Role of Vitamin C Against Cypermethrin Induced Toxicity in *Labeo rohita* (Ham.): Biochemical Aspects

By

SANA ULLAH

Department of Animal Sciences

Faculty of Biological Sciences

Quaid-i-Azam University

Islamabad

2015

Protective Role of Vitamin C Against Cypermethrin Induced Toxicity in *Labeo rohita* (Ham.): Biochemical Aspects

A thesis submitted in partial fulfillment of the requirements for the Degree of

MASTER OF PHILOSOPHY

IN

FISHERIES AND AQUACULTURE

By

SANA ULLAH

Department of Animal Sciences

Faculty of Biological Sciences

Quaid-i-Azam University

Islamabad

2015

Declaration

I hereby declare that the work presented in the following thesis is my own effort, except where otherwise acknowledged, and that the thesis is my own composition. No part of this thesis has been previously presented for any other degree.

SANA ULLAH

CERTIFICATE

This dissertation "Protective Role of Vitamin C Against Cypermethrin Induced Toxicity in *Labeo rohita* (Ham.): Biochemical Aspects" submitted by **Mr. Sana Ullah** is accepted in its present form by the Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirement for the degree of Master of Philosophy in Fisheries and Aquaculture.

Supervisor:

Dr. Amina Zuberi

External Examiner:

Dr. Muhammad Afzal
PSO/PL
Fisheries Aquaculture & Fisheries
Program, NARC, Park Road,
Islamabad

Dr. Sarwat Jahan
Chairperson

CHAIRPERSON
Dept. Of Animal Sciences
Quaid-i-Azam University
Islamabad

Date: 16.09.2015

DEPARTMENT OF ANIMAL SCIENCES
Date 16-9-15

Affectionately Dedicated to:

My loving mother, brothers, sisters and their children

Table of Contents

List of Abbreviations

ADP	Adenine dinucleotide phosphate
BSA	Bovine serum albumin
CAT	Catalase
CYP	CYP
EDTA	Ethylene diamine tetra acetic acid
GSH	Total reduced glutathione
GSH-Px	Glutathione peroxidase
GR	Glutathione reductase
GSSG	Oxidized state (Glutathione)
GST	Glutathione-S-transferase
H_2O_2	Hydrogen peroxide
hr	Hours
LPO	Lipid peroxidation
NAD	Nicotinamide adenine dinucleotide
NaH_2PO_4	Sodium dihydrogen phosphate
O_2^-	Superoxide
PBS	Phosphate buffer saline
POD	Peroxidase
ROO	Peroxy radicals
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TBARS	Thiobarbituric reactive substance
TCA	Trichloroacetic Acid
M	Molar solution
µmol	Micromole
mM	Millimole
nmol	Nanomole
Kg	Kilogram
mg	Milligram
ng	Nanogram

LIST OF TABLES

List of Figures

Acknowledgements

Praise is to Allah, Lord of the Worlds. The Most Beneficent, the Most Merciful, Who is the entire source of knowledge and wisdom endowed to mankind; Who gave me courage and potential to pursue this goal Whom I believe that He never spoils any effort of good deeds. Blessings of ***Allah*** *be upon His* ***Prophet Muhammad (PBUH)****", the city of knowledge and blessing for entire creature, who has guided his* ***Ummah*** *to seek knowledge from Cradle to Grave, and enabled me to win honour of life.*

It is a matter of great pleasure to express my sincere regards to my honourable supervisor Assistant Prof. ***Dr. Amina Zuberi****, Department of Animal Sciences, for affectionate supervision, inspiring attitude, masterly advice and encouragement. Without her useful intellectual suggestions, it would have been impossible for me to complete this tedious work.*

I express my gratitude to Prof. ***Dr. Wasim Ahmad****, Dean Faculty of Biological Sciences and Prof.* ***Dr. Sarwat Jahan****, Chairperson Department of Animal Sciences, for providing me all the privileges during my research work.*

I must acknowledge my deepest debt of gratitude to my teachers from Department of Animal Sciences, QAU Islamabad and Department of Zoology, University of Peshawar, KP.

I feel highly privileged to express my profound gratitude to my dear friends ***Hizb Ullah Wazir, Tariq Iqbal Khan and Asad Aryan (Reproductive Physiology Lab.)*** *for their devotion, creativity, affectionate criticism and keen interest in my work.*

I wish to express my deep and sincere gratitude to my captain ***Imdad Ullah Salar*** *for his encouragement and personal guidance. My heartfelt thanks to my seniors* ***Shahzad Ahmad, Kifayat Ullah, Sajjad, Muhammad Rauf, Baber Khan, Ahmad Shoaib, Ibrar Muhammad, Naima Younus, Shams Un Nihar, Saba Rauf, Imrana Amir*** *and* ***Muhammad Ahmad****, and dear class fellows* ***Layaq Jan, Muhammad Iqbal*** *and* ***Hidayat Ul Haq*** *for their care and moral support.*

I must acknowledge my debt to my junior lab fellows ***Sana Ullah, Fawad Aziz, Karim Jauhar Khan, Muhammad Akmal, Awal Syed, Allaud Din Khan, Hussain Ahmad, Azka Anwar, Tayyaba*** *and* ***Zubaira Sultan*** *for their kind help and cooperation during my research work. I am also thankful to them for their nice company and time they provided me with beautiful memories that I will treasure throughout my life.*

I am obliged to ***Yasir Sultan, Naeem Masih*** *and* ***Sami Ullah*** *for their help, support and motivation during my research.*

My heartfelt thanks to my friends ***Naseer Ullah*** *(Parasitology Lab.),* ***Usman Khan*** *(Department of Anthropology),* ***Khaista Rahman, Lutfur Rahman, Jalal Shah*** *(Department of Biotechnology),* ***Tanveer Ahmad, Wasim Akram, Hayat Dirvi*** *(Department of Microbiology),* ***Khadim Shah*** *(Department of Biochemistry), and other fellows from faculty of Biological Sciences for their nice company, cooperation and help during my research work.*

Words always seem to shallow whenever it comes to my beloved mother for her constant love, care and endless support. All my love to my little angels ***Ameema Inam, Habiba Inam, Faiza Inam, Furqan Ullah, Irfan Ullah, Hassan Zia*** *and* ***Layah Inam****. I owe my heartfelt thanks to my elder brothers. My special gratitude is due to them for providing me a chance to prove and improve myself through all walks of life. I am honoured to have them as my elder brothers. My younger brothers deserve special mention for their incredible care, love, respect and prayers.*

A non-payable debt to my loving mother and family, their wish motivated me in striving for higher education; they prayed for me, shared the burden and made sure that I sailed through smoothly.

I would like to thank my sisters and brothers-in-law for prayers and support during my entire student life.

I am thankful to all those who helped me.

SANA ULLAH

ABSTRACT

Cypermethrin (CYP) is used as a substitute to organophosphates and organochlorines for controlling pests on account of its low toxicity and less environmental persistence. The current study was conducted to evaluate the toxic impacts of CYP on biochemical indices of rohu, *Labeo rohita* (Hamilton, 1822) and the protective role of vitamin C on CYP induced toxicity. The acute toxicity of CYP was found out first. Then, further study was undertaken for assessing the effect of LC_{50} of CYP on behaviour, total protein content and lipid peroxidation in different tissues, antioxidant defence system, protein metabolic enzymes, digestive enzymes and DNA damage induced in the peripheral blood erythrocytes. Furthermore, the ameliorative role of vitamin C at two dietary levels (100 and 200 mg/Kg diet) against CYP induced toxicity was also assessed. For finding acute toxic concentration of CYP, fingerlings of rohu were exposed for 96 hours to seven different concentrations of CYP ranging from 3 to 6µg/L in a static culture system. The LC_{50} for 96 hr was calculated by arithmetic method and verified by probit analysis and Dragstedt-Behren's equation and found to be 4.5 µg/ L. For toxicological study, fingerlings of rohu were distributed into four groups. Group 1st served as a control, fed 35% protein basal diet and was not exposed to CYP, while Group 2nd was fed basal diet and exposed to CYP. Moreover, Group 3rd and Group 4th were fed diets supplemented with vitamin C at the rate of 100 and 200 mg/kg diet respectively and exposed to CYP. Fingerlings were reared on basal and Vitamin C supplemented diet for 28 days prior exposure to CYP and then after exposure to pesticide at different time intervals, blood, brains, gills, liver, muscle and intestine tissues were collected from different group of fingerlings for biochemical analysis. Results indicated the time dependent significant decrease in total protein contents whereas a significant increase in LPO level , activities of antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST)], activity of digestive enzymes (protease, lipase and cellulase) and protein metabolic enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glutamate dehydrogenase (GDH), and lactate dehydrogenase (LDH)] in the studied tissues after exposure to an acute concentration of CYP. Maximum reduction was observed in liver as compared to other studied tissues of rohu, suggesting the maximum defensive mechanisms of liver against oxidative stress. Moreover, a time dependent increase in DNA damage was also observed in terms of tail length, % DNA in tail,

tail movement and olive tail movement after exposure to LC_{50} of CYP. However, statistically comparable results of all biochemical analysis in both Group 4th (fish fed high level of vitamin supplementation i.e. 200 mg/Kg diet before exposure to CYP and Group 1st (fish reared on basal diet and not exposed to CYP), indicating the protective role of vitamin C against CYP induced toxicity. Thus, the results of the present study revealed the effectiveness of vitamin C as a feed additive for countering CYP induced toxicity in *L. rohita*. Moreover, this study supports the view of using fish oxidative stress, antioxidant enzymes status and induced genetic damage as biomarkers in aquatic toxicological studies and diagnosing pollution.

INTRODUCTION

INTRODUCTION

The present era of green revolution that is witnessing a swift increase in human populations across the globe is apparently depicting humans' dependency on the available natural resources (Espinoza-Qui˜nones et al., 2005; Ullah et al., 2014a). The current scenario has led to efforts for technological advancements to cope with the need of societies. This in turn ensued by evolving ever increasing dissolution of different synthesized chemicals in the environment specifically aquatic bodies that are being used as dumping sites in most parts of the world (Meitei et al., 2004; Jabeen et al., 2011; Ullah et al., 2014b). Although the pollution phenomenon is very much complex but luckily its sources are as evident as bright day-light in almost all regions across the globe (Ullah et al., 2014c). Pollution has been the key universal off-putting factor for men, which has been worsened by the hasty growth of human populaces and instantaneous industrialization (Ullah et al., 2014d; Sthanadar et al., 2015).

Water Pollution

Pakistan is facing the continuously deteriorating problem of pollution, explicitly water pollution (Ali et al., 2013). It has been defined variously, usually comprises of degradation resultant of different anthropogenic activities consequently rendering water as unfeasible for various intended purposes (Agarwal, 2002). Although some natural marvels also evolve natural ecologies such as eruptions of volcanoes, mud sliding etc. but human activities is characteristically very rapid and insistent that surpass the evolutionary prospective of ecosystem, resulting in their deteriorated state (Ullah, 2014).

Water beside an essential component of human life and existence, is also a key to sustainability of society and the nation (Deshpande and Aher, 2012). It is the central core of different human activities such as agriculture, industries, environmental and recreational activities, and domiciliary activities (Malik et al., 2012; Ghorbani et al., 2013). It simply means that water is universal in almost all practices of human life (Prasad and Narayana, 2004). Unfortunately due to increase in water pollution, it has been projected that approximately 2.7 billion populaces will face its shortage by 2025 (UN, 2003). Pollution of water is a serious threat to life existence on the planet therefore efforts should be made on priority basis for eliminating pollution sources and restoring the damaged ecosystems back (Yousaf et al., 2013).

Nowadays one of the main objectives and tasks for human beings is to secure relief of hunger by producing and growing more edibles which is the basic need of life. Currently Pakistan is affianced in the massive mission of feeding the gigantic population of more than 180 million individuals (USCB, 2015). At the same time controlling economically important or public health wise hazardous pests such as insects, fungi and weeds etc. is of prime importance and focus. This task was almost impossible, but 1960's green revolution resulted in making us self-sufficient in producing some basic life commodities and satisfactory food for nourishing Pakistan's teeming population. On contrary pesticides use has seriously elevated some major issues regarding pollution (Gagnaire et al., 2004; Jain et al., 2005; Mustapha, 2008; Naeem et al., 2010; Abu-Darwish et al., 2011).

Pesticides' Pollution

Pesticide is a term used for a large number of compounds having varying biological activities and chemical natures that are clustered together because of their capability to abolish or eradicated pests (Gupta, 1989; Wood et al., 2000). According to United States' pesticides control act, Pesticides are all those substances or their mixture used for prevention, destruction, repelling, deterring, resisting or mitigation of pests (Marigoudar, 2012).

Water pollution due to pesticides may be due to directly apply these for controlling aquatic flora or seepage from agricultural lands through soil (Kosygin et al., 2007; Sarkar et al., 2008). These may also lead to water bodies through agricultural runoffs, which may be due to surface water of transportation through air (Aprile and Bouvy, 2008; Arimoro and Ikomi, 2008; Shanbehzadeh et al., 2014). In order to elevate yield with low labor and in less time, a huge quantity and variety of pesticides have been manufactured and still being under development (Ullah and Zorriehzahra, 2014). These are widely spread in both urban and agriculture landscapes (Fetoui et al., 2010). This simply clarifies pesticides' residues or pesticides as a major contributor of water pollution (Murthy et al., 2013).

Across the globe different pesticides are used in different ratio such as insecticides account for 80% of the total pesticides, herbicides for 15% and fungicides for 1.46% (Marigoudar, 2012). Different types of insecticidal chemicals are used around the world, which are well documented (Johnson and Finely, 1980; Hart and Pimentel, 2002; UK Pesticides Action Network, 2009). Of these insecticides, the most commonly employed ones are pyrethroids (Friberg-Jensen et al., 2003; Kaviraj and Gupta, 2014).

Pyrethroids

The Caucasian tribes used flowers of pyrethrum (Genus: Chrysanthemum) as a controlling agent for body lice in early the 18th century. The insecticidal potentials of pyrethrum flower (*Chrysanthemum cinerariafolium*) was recognized in the mid of last century while in 19th century introduced to United States, Europe, Africa and Japan. Currently Kenya ranks 1st in pyrethroids production followed by Japan, Congo, Uganda and Tanzania (Marigoudar, 2012). Currently there are more than thousand potent and broad spectrum insecticides enlisted in this group of pesticides (Fetoui et al., 2008). Many scientists have compiled different types of pyrethroids and have divided them into different major classes on account of their mode of action, persistence, severity of action and toxicity to non-target organisms such as Kaviraj and Gupta (2014) have comprehensively reviewed type II synthetic pyrethroids, Cypermethrin being one of these pyrethroids. Marigoudar (2012) comprehensively compiled various uses, chemistry and mode of action, toxicities, persistence and bioaccumulation factors of different pyrethroids. Ullah and Zorriehzahra (2014) systematically reviewed various types of toxicities caused by different pyrethroids pesticides in different species of fish.

Cypermethrin (CYP)

Cypermethrin [(RS)-cyano-(3-phenoxyphenyl) methyl-(IRS)-cis -Trans-3-(2, 2 dichloroethenyl)-2, 2-dimethyl-cyclopropane carboxylate] is one of extensively used and highly effective synthetic cyanophenoxybenzyl pyrethroids (Saha and Kaviraj, 2009). It is synthetically derived from natural compounds, itself isolated from Chrysanthemum flower, *Chrysanthemum cinerariaefolium* (Roy, 2002). Cypermethrin (CYP) is extensively employed in almost all type of agriculture, buildings, forestry, gardens and farmyards for insects' prevention (Khan et al. 2006; Casida et al., 1983). CYP is commercially used to prevent cotton pests and soyabean pest (WHO 1989; Carriquiriborde et al. 2007). Its use for repelling and controlling mosquitos has proven to be very effective in preventing malarial parasites (Tananchai et al., 2012). But tragically CYP is misused and illegally employed in different parts of the world for fishing purpose, such as Pakistan (Nafees and Jan, 2009) while in some parts of the world, it is being utilized as a chemotherapatic agent by aquatculturists in order to control copepods' parasitic infections (Athanassopoulou et al., 2009; Medina et al., 2002; Medina et al., 2003). Therefore this large scale application of CYP is resulting in major

threats to aquatic ecology and aboding organisms such as fish (John and Prakash 2003; Oudou et al. 2004).

Cypermethrin as Toxicity Inducer in Fish

Cypermethrin induces various types of toxicities in fish and in severe concentration it does lead to huge fish mortalities (USEPA, 2000; Narra et al., 2015). Some of these toxic effects in the form of changes in different behavioral, physiological, anatomical, hematological, biochemical, enzymatic, hormonal and molecular aspects are briefly reviewed here.

Behavioral Changes

Studies have revealed that CYP induces various types of behavioral changes in different fish species. Cypermethrin induced toxic effect on behavior of *Labeo rohita* and led to sinking in bottom, hyper excitability, equilibrium loss, irregular and erratic swimming and movements and darting swimming movements (Marigoudar *et al.*, 2009). *Tor putitora* in response to CYP showed different changes in behaviour such as adaptation of vertical position, motionlessness, sluggishness, abrupt swimming, loss of equilibrium, elevated air gulping, loss of balance, increased surface activity and jumping (Ullah *et al.*, 2014e). Cypermethrin in combination with Chlorpyriphos induced behavioural changes in *Poecilia reticulate* (Wast et al., 2014). Cypermethrin changed behaviour of *Rhamdia quelen* such as balance loss, spiral movement, sudden swimming, upright swimming, dyspnoea and swimming alterations (Montanha et al., 2014).

Histopathological Changes

Cypermethrin induced histopathological changes in brain (spongiosis and neuronal degeneration), kidney (pyknotic nuclei swollen and exfoliation), liver (necrosis and hepatocytes vacuolation) and gills (epithelial lifting, haemorrhage, cellular infiltration and filaments' cells proliferation) of *Clarias gariepinus* (Ayoola and Ajani, 2008). In *Tor putitora* it caused histopathological alterations of brain (severe spongiosis, infiltration, neuronal degeneration, and discolouration), gills (damaged gills, hetrophilic infiltration, swollen gill filament, congestion and cellular infiltration) and liver (hepatic necrosis, fatty infilteration, congestion, haemorrhage vacuolation and glycogen vacuolation) (Ullah *et al.*, 2014f).

Haematological Changes

Cypermethrin exposure decreased haemoglobin concentration, total erythrocyte, mean corpuscular haemoglobin concentration while increased mean corpuscular haemoglobin and mean corpuscular volume in *Prochilodus lineatus* (Parma et al., 2007). In *Clarias gariepinus* CYP significantly reduced Packed cell volume, Red blood cells, Haemoglobin, leucocytes, lymphocytes, eosinophils, monocytes, thrombocytes, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume while elevated white blood cells, neutrophils, and erythrocyte sedimentation rate (Akinrotimi et al., 2012). CYP decreased RBCs count while WBCs count increased with time in *Tor putitora* (Ullah *et al.*, 2014e). CYP exposure caused elevation in white blood cells counts, mean corpuscular haemoglobin levels, haematocrit, Haemoglobin content, granulocytes, mean corpuscular volume and erythrocytes while reduced lymphocyte count, mean erythrocyte haemoglobin and mean erythrocyte volume in *Catla catla* (Kannan et al., 2014). CYP also altered haematology of different other fish species such as *Labeo rohita* (Adhikari *et al.*, 2004), *Onchorhynchus mykiss* (Atamanalp et al., 2002), and *Rhamdia quelen* (Montanha et al., 2014), *Cyprinus carpio* (Masud and Singh, 2013).

Changes in Enzymes Activities

CYP inhibited acetylcholine esterase activity in *Labeo rohita* significantly, especially in brain, then in muscles, gill and liver (Marigoudar *et al.*, 2010). CYP inactivate Acetylcholine esterase (AChE), which led to acetylcholine accumulation in cholinergic synapses, finally leading to hyperstimulation (Marigoudar *et al.*, 2009; Marigoudar *et al.*, 2010). Cypermethrin significantly induces changes in AChE, lactic dehydrogenase and succinic dehydrogenases' activities in nervouss tissues of *Colisa fasciatus* (Singh *et al.*, 2010). Cypermethrin exposure caused significant alterations in the activities of different enzymes including cytochrome oxidase, acetylcholinesterase, alkaline phosphateses, acid phosphatases, aspartate aminotransferase, alanine aminotransferase and protease in muscle and liver tissues of *Labeo rohita* (Tiwari et al., 2012). CYP induces significant changes in oxidative system of fish. It induced very much alteration in antioxidant enzyme such as peroxidases, glutathione reductases, lipid peroxidases and catalases got increased in different tissues of *Tor putitora* including gills, liver, brain and muscles (Ullah *et al.*, 2014e). It also induced changes in free amino acids, protein carbonyls, malondialdehyde, hydrogen peroxide and catalases in liver of *Labeo rohita* (Marigoudar *et al.*, 2012). CYP increased the activities

of glumate dehydrogenases and transaminases (Begum, 2007). CYP changed alkaline phosphatase, gamma glutamyltransferase, aspartate aminotransferase and alanine transaminase activities in *Rhamdia quelen* (Montanha et al., 2014). CYP greatly reduced total magnesium adenosinetriphosphatase, sodium-potassium adenosinetriphosphatase, and adenosinetriphosphatase activities in the gill of *Catla catla* (Vani et al., 2012). CYP also showed greater reticence of total, sodium, potassium and magnesium ATPase enzymes while increase glycogen phosphorylase level in *Clarias batrachus* (Begum, 2009). CYP also decreased alkaline phosphatases activity in *Heteropneustes fossilis* (Saha and Kaviraj, 2009) and *Labeo rohita* (Das and Mukherjee, 2003) while some studies reported increase of alkaline phosphatases after exposure to Cypermethrin (Jee et al., 2005; Borges et al., 2007; Loteste et al., 2013).

Molecular Changes

Cypermethrin induced significant changes in nucleic acids (DNA and RNA) in gonads of *Colisa fasciatus* (Singh *et al.,* 2010). CYP elevated p53 gene expressions and downregulated ogg1 as well as increased caspase-3 activity in embryos of zebrafish which resulted in apoptosis and oxidative stress (Shi et al., 2011).

Protein Contents and Glucose

Cypermethrin exposure led to significant reduction of proteins content in *Labeo rohita* (Veeraiah and Durga-Prasad, 1998), *Colisa fasciatus* (Singh *et al.,* 2010) and decreased protein contents in different tissues of *Tor putitora* (Ullah *et al.,* 2014e). Cypermethrin induced a dose and time dependent change in lactate level, pyruvate, glycogen, nucleic acids, amino acids and total protein in *Labeo rohita* (Tiwari et al., 2012). CYP exposure increased liver glycogen, muscle glycogen level and plasma glucose while decreased plasma proteins in *Catla catla* (Kannan et al., 2014). CYP increased ammonia content and free amino acid while decreased protein contents in *Clarias batrachus* (Begum, 2007). CYP also changed plasmatic protein in *Rhamdia quelen* (Montanha et al., 2014). CYP reduced hepatic glycogen accompanied by rise in blood glucose, thus induced hyperglycaemia in different fish species including *Sebastes schlegeli* (Jee et al., 2005), *Onchorhynchus mykiss* (Velisek et al., 2006), *Labeo rohita* (Das and Mukherjee, 2003), and *Heteropneustes fossilis* (Saha and Kaviraj, 2009). CYP also resulted in reduction of hepatic

glycongen in *Clarias batrachus* (Begum, 2005) and *Oreochromis mossambica* (Reddy and Yellamma, 1991).

Vitamin C (Ascorbic Acid, AA) for Amelioration against Cypermethrin

As stated earlier regarding toxicities induced by Cypermethrin in different fish species, Vitamin C (Ascorbic Acid) in the present study is used as Amelioration feed ingredient against cypermethrin induced biochemical toxicities. Vitamin C is being employed in this study because of its direct correlation with fish health, fish immune system, fish growth and maintenance, capillary fragility, skeletal deformation, speedy wound healing, providing strong resistance to stress in fish specifically pesticides stress, oxidative damage and protection against DNA damage (Ortuno et al., 1999; Datta and Kaviraj, 2003; Lin and Shi-Yen, 2005; Garcia et al., 2007; Misra et al., 2007; Vani et al., 2011; Zhou et al., 2012; Sram et al., 2012). The second reason of selecting Vitamin C for this study is the sensitiveness of fish towards an optimum level of Vitamin C as fish are unable of its *de novo* synthesis and its involvement in fish' self-defense mechanism (Dabrowski, 1990; Ai et al., 2004; Saha and Kaviraj, 2012).

Vitamin C is having different potentials but one of the key functions is to quench, reduce and satiate oxygen radicals that are arising in result of cellular respiration. Beside this in phagosomes respiratory burst activities led to the production of hydrogen peroxide, hydroxyl radicals, and superoxide anions etc. (Hurst and Barrette, 1989). Production of these radicals (free) impose oxidative stress on proteins (sulphydryl groups), thus promoting destabilization of lipids in the membrane (Hardie et al., 1991). There are other wide varieties of functions of Vit C such as cofactor for synthesis of collagen, hexose monphoshpate shunt modulator, water soluble redox reagent, steroid synthesis regulator, and also inactivates hepatic microsomal hydroxylases (Panush and Delafuente, 1985).

Protective Role of Vitamin C

Vitamin C is a strong non-enzymatic antioxidant and is the most significant scavenger of free radical specifically in extracellular fluids (Fetuoi et al., 2009). Vitamin C uses to trap radicals in aqueous phases and protect bio-membrane from damage of peroxidation (Niki et al., 1995; Yavuz et al., 2004; Sulak et al., 2005). It is an election donor, thus protects the body from pollutants and radicals (Iqbal et al., 2004; Banerjee et al., 2001). Traber and Stevens (2011) have comprehensively reviewed the beneficial effects of Vitamin C and E

against certain disorders from mechanistic perspectives while Sram et al. (2012) systematically reviewed protective role of Vitamin C against DNA damage. Villacorta et al. (2007) reviewed the antioxidant and non-antioxidant characteristics of Vit C in detail. A very rich and huge amount of literature is available on protective role of Vitamin C against different toxicants including heavy metals, pesticides and such type of other chemicals induced toxicities in different fish species (Durve and Lovell, 1982; Li and Lovell, 1985; Wahli et al., 1986; Liu et al., 1989; Navarre and Halver, 1989; Meier et al., 1991; Dunier et al., 1995; Verlhac et al., 1998; Ortuno et al., 1999; Montero et al., 1999; Ortuno et al., 2001).

Scope of the Present Study

Cypermethrin was selected as a toxicant for conducting this study on account of the accepted fact that it is a widely used pesticide across the world. Cypermethrin leads to aquatic bodies worldwide which were confirmed by a monitoring program in 1998, discovering that 20% of the inquired sites were having cypermethrin in a concentration higher than permissible concentration and were falling in the range of 0.078 µg/Lto 2.8 µg/L(Pfeuffer, 1999). The study conducted by Laabs et al. (2002) revealed the presence of cypermethrin in rainwater at 0.376 µg/Lconcentration. From the available literature it is widely known and confirmed that cypermethrin concentration is higher than the permissible range in water bodies which can be harmful to all forms of aquatic life.

In Pakistan, cypermethrin is used on a wider range of crops such as wheat, sugarcane, brinjal, okra, cabbage, cotton and sunflower. It is also thought that cypermethrin is an immobile and did not expect to be biomagnifying through food chain. On account of its property of higher lipophilicity, it is having a higher rate of absorption (Michelangeli et al., 1990; Fahmy and Abdallah, 2001; Celik et al., 2003, 2005; Fetoui et al., 2008). This renders fish as the most subtle, penetrating and sensitive to cypermethrin (Saha and Kaviraj, 2009).

Labeo rohita commonly known as rohu inhibits freshwater bodies naturally but is of the most extensively cultured fish species in Pakistan. It is one of the greatly edible and highly cultured Indian major carps thus contributing to national economy. Despite being widely available in rivers, reservoirs, ponds and tanks, this teleostian species is vastly acquiescent to laboratory environment thus representing widespread prevalence of cyprinids.

Fish is generally considered as sentries of bio indication for water pollutants and vital investigational model in eco-toxicological experiments (Fazio et al., 2014). Therefore owing

to this view, rohu (*Labeo rohita*, Hamilton) was selected and considered for the current study. Rohu belongs to pyhylum Chordate, sub phylum Vertebrata, division Gnathostomata, super class Pisces, class Osteichthyes, super order Teleostei, order Cypriniformes and family Cyprinidae.

Rationale of the Current Investigation

In the present day sciences, it has been very necessary to interpolate the impacts of chemicals and pollutants on living organisms revealed by experiments in laboratory condition to community and population levels. There is a need for evaluating biochemical indicators of that organisms' health and ill effects of that toxicant. By studying these indicators the environmental glitches can be possibly identified and recovered before some serious alterations. For evaluating exposure as well as the impacts of a contaminant, biochemical alteration in the organism can serve as a key marker. In other words changes induced in biochemical system of the organisms will depict the ill effects of that chemical on ecosystem. Changes in biochemical system are considered as very sensitive pointers, even more sensitive than those at advanced side of biological complexities. These changes at molecular basis trigger alterations at organization level such as response can show the impact at organs' levels.

Objective of the Current Study

Ample studies regarding toxicological aspects of different regionally and locally used toxicants, pollutants or chemicals on indigenous species will not only aid protecting the environment but also reveal eco-toxicological data that can be employed for supervisory affairs of these toxicants in that specific zone or somewhere else. Keeping in mind the same very view, the present study was aimed:

- To evaluate the toxic effects of cypermethrin in biochemical indices in rohu, *Labeo rohita* and the protective role of Vitamin C against these biochemical changes.

The hypothesis for the current study was:

- Vitamin C ameliorates the biochemical changes induced by Cypermethrin in rohu, *Labeo rohita*.

To achieve the anticipated objective the following parameters were included in the study and were assessed.

- ✓ Toxicity evaluation of cypermethrin against rohu.
- ✓ Behavioral changes induced by cypermethrin in rohu and protection provided by Vitamin C.
- ✓ Cypermethrin induced changes in total protein contents in brain, gills, liver and muscles of rohu and protective role of Vitamin C against these changes.
- ✓ Changes induced in activities of antioxidant enzymes including Catalase (CAT), Peroxidase (POD), Superoxide Dismutase (SOD), Glutathione Reductase (GSR), Lipid Peroxidase (LPO), Total Glutathione contents (GSH), Glutathione Peroxidase (GSH-Px) and Glutathione-S-Transferase (GST) in brain, gills, liver and muscles of rohu and protection provided by Vitamin C.
- ✓ Induced changes in enzymes of protein metabolism e.g., Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Glutamate Dehydrogenase (GDH) and Lactate Dehydrogenase (LDH) in liver, muscles and gills of rohu and ameliorative role of Vitamin C against these changes.
- ✓ Changes induced in activity of Digestive enzymes i.e. Protease, Cellulase and Amylase activity and Vitamin C provided protection.
- ✓ DNA damage caused by cypermethrin and ameliorative role of Vitamin C against cypermethrin for inducing DNA damage.

MATERIALS AND METHODS

MATERIALS AND METHODS

This experiment was undertaken at Fisheries Research Station, Department of Animal sciences, Quaid-i-Azam University Islamabad.

Test Animals' Transport and Acclimatization

A total of 250 uniform sized, active and healthy fingerlings of rohu, *Labeo rohita* (average weight 6.55±1.01 g and average length of 8.17±0.794 cm) were collected from Faisalabad Fish Hatchery and were transported to Fisheries Research Station, Department of Animal Sciences, Quaid-i-Azam University Islamabad through close system live hauling method. Prior shifting fish to circular tanks (fiberglass) from oxygen bags, tempering was carried out by steady and regular mixing of water from circular tanks in polyethylene bags. In order to avoid any damage to the fish, on account of jumping due to journey stress, special care was taken. The tanks having dechlorinated and well oxygenated water were given protection with nets. Then after two days the fish were shifted to aquaria (60 x 30 x 30 cm), previously washed with $KMnO_4$ in order to free its walls from growth of microbes. The fish were acclimatized for 15 days in these glass made aquaria.

During the experimental trial period, water was exchanged on regular basis daily and a 12:12 hr of photoperiod was sustained during the test periods and acclimation. While acclimatizing fish, they were fed to satiation with 35% protein based diet (Table 1) at the rate of 5% of their body weight with small dried pellets twice a day. Remains of the feed and excretory waste were siphoned off daily to avoid stress on the fish. The aquarium was fit with air stones for continuous aeration. The water quality factors were assessed on regular basis to ensure their optimum range prevalence. To avoid water quality deterioration, dead fish were removed as quickly as possible. During the trials period ammonia was lying under 0.25 ppm whereas Temperature was 25.8±1.2°C, pH was 7.9±0.4 at 26°C, hardness was 300 mg/L and dissolved Oxygen was ranging from 6.9 to 7.5 mg/L.

Experimental Toxicant - Cypermethrin

Cypermethrin (Cyano (3-phenoxyphenyl) methyl 3-(2, 2-dichloroethenyl)-2, 2-dimethyl-cyclopropane-carboxylate) of Sigma-Aldrich (100 mg/ 5 ml in solution form), purchased from AMS Traders, Islamabad Pakistan, was used for preparing stock solution.

CYP stock solution was prepared in acetone (80%), as it is non-toxic to fish. Required quantity of CYP was used in preparing stock solutions.

Table 1. Formulation of basal diet for rohu, *Labeo rohita* (35% protein)

Ingredients	Amount (g kg^{-1})
Soybean meal	212
Sunflower meal	212
Fish meal	105
Gluten 30%	105
Canola meal	212
Rice polish	52
Dicalcium phosphate (DCP)	10
Carboxy methyl cellulose (CMC)	10
Vitamin premix [a]	20
Wheat bran	52
Vegetable oil	10

[a] (Vitamin premix contains vitamins, amino acid and minerals premix kg^{-1})

Vitamin AB.P 40,000,000 IU, Vitamin K3B.P 800 mg, Vitamin D3B.P 820,000 IU, Vitamin EB.P 6200 mg, Vitamin B3B.P 5100 mg, Vitamin B2B.P 2500 mg, Vitamin B12B.P 1000 mg, Vitamin PP B.P 10,500 mg, DL- Methionine B.P 50,500 mg, L. lysine B.P 10,500 mg, Manganese USP 30,000 mg 15,100 mg, Zinc USP 17,555 mg, Copper B.P 1000 mg, Choline chloride USP 125,500 mg, Cobalt B.P 50 mg, Iodine B.P 300 mg, Selenium B.P 80 mg.

Preparation of CYP solution

Stock solution of Cypermethrin was prepared by dissolving 0.5 ml pesticide in 9.5 ml of acetone (80%) to make 10 ml stock solution. As 5 ml solution form of CYP contains 100 mg CYP, so making the above mentioned solution mean 10 mg CYP in 10 ml or in other words 1 µg CYP in 1 µl. The flask used for solution preparation was shaken well to get

homogenous solution. This stock solution was used to make further concentrations used for toxicity tests/ evaluating toxic concentration of CYP to rohu, *Labeo rohita*.

Toxicity Test for Acute Concentration of CYP

Median tolerance limit (LC_{50}) of the fish, rohu to CYP for 96 hr was determined in laboratory through semi-static method. The fish was distributed in glass aquaria, 10 fingerlings in approximately 20 liter water per aquaria for toxicity evaluation. For finding the concentration causing 50% motility, the fish were exposed to seven different concentration of CYP i.e. 3 µg/L, 3.5 µg/L, 4 µg/L, 4.5 µg/L, 5 µg/L, 5.5 µg/L and 6 µg/L based on available literature on different carps. For determining LC_{50}, fish mortality was noticed every 24 hr up to 96 hr while removing dead fish to avoid water contamination. The LC_{50} was estimated with a confidence limit of 95% for CYP for 96 hr through probit method (Finney, 1971), recommended as standard statistical method by OECD (1996) for data analysis regarding evaluation of pesticides' toxicity. Further verification was done through Dragstedt-Behren's equation/method followed by Vidyunmala *et al.* (2010). The concentration 4.5 µg/L was found as median lethal concentration (LC_{50}), causing 50% mortality for 96 hr. Figure 1 is showing % mortality conversion to probits' units.

Table 3.2 Transformation of percentages to probits

%	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
—	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Experimental Design

Semi-static closed system was used for conducting this experiment. Healthy, active and uniform size fish irrespective of sex were chosen and equally distributed in twelve glass

aquaria having a size of 60 x 30 x 30 cm at a stocking density of 1.5 kg/m^3. The experiment was carried out in replicates comprising of four groups, such as the first three aquaria served as control group while the rest nine aquaria were different experimental groups e.g. the second group feed with control feed and exposed to CYP, the third group fed with Vitamin C (100 mg/kg diet) and exposed to CYP and the fourth group fed with Vitamin C (200 mg/kg diet) and exposed to CYP.

After 28 days of feeding trials, the fish in the three groups except control were exposed to acute concentration, 4.5 µg/L of CYP while group first (control group) received 80% acetone equal to CYP volume used in treatment groups. The water of all aquaria was changed after every 24 hr and CYP concentration was restored afresh. The experiment was undertaken for 96 hr (4 days). After every 24 hr such as 24, 48, 72 and 96 hr, 5 fish of each aquarium were captured before exchange of water. Blood was obtained from caudal vein of fish in EDTA tubes for evaluating DNA damage through comet assay and was stored at 4°C. These fish were stunned to death and using sterilized instruments, their target tissues including brain, gill, liver, intestine and muscles tissues were dissected out. They were kept in Ziploc bags and frozen in liquid nitrogen. Then they were stored at -20°C for protein estimation, antioxidant enzymes assays and digestive enzyme analysis. The following tissues were selected for the giver parameters of the study.

- **Protein estimation:** Brain, Gills, Liver, Muscles
- **Antioxidative enzymes:** Brain, Gills, Liver, Muscles
- **Digestive enzymes:** Intestine
- **Protein metabolism:** Gills, Liver, Muscles
- **DNA damage assessment through Comet Assay:** Blood

Behavioural Study

After exposure to acute concentration of CYP, fish behaviour in treated groups were observed keenly and was compare with that to unexposed Control group. Every visible behaviour of fish was checked such as equilibrium state, beating of operculum, movement, jumping, aggression and hyper or hypo activeness etc.

Assessment of Tissue Biochemical Studies

Protein Estimation

Total protein content in liver, brain, gills and muscle tissues were determined by Lowry's method (Lowry et al., 1951). Tissues of each of organ, weight 90 mg, were taken

and homogenized in phosphate buffer and were centrifuged at 10,000 rpm for 20 min at 4°C. Briefly, the standard bovine serum albumin (BSA) stock solution (1mg ml^{-1}) was prepared by dissolving 15 mg BSA in 15 ml distilled water. Then solution No. 1 was prepared by taking 50 ml of 2% sodium carbonate (Na_2CO_3) and mixed with 50 ml of 0.1 N NaOH solutions. After this solution No. 2 was prepared by mixing of 10 ml of 1.56 % copper sulphate solution with 10 ml of 2.37 % sodium potassium tartarate solution. Folin-Ciocalteau reagent solution was freshly prepared on the day of use by mixing 2 ml of commercial reagent with an equal volume of water.

Alkaline solution was prepared by mixing 2 ml of solution No. 2 with 100 ml of solution No.1. The sample was thawed and 1ml of alkaline solution was mixed with 0.1ml sample. The mixture was incubated for 10 min. Then 1:1 Folin-Ciocalteau phenol reagent was added to each tube and vertex to mix thoroughly. After 30 min of incubation optical density was noted at 595 nm by using spectrophotometer and concentration of soluble protein was calculated using bovine serum albumin standard curve.

Extraction of antioxidant enzymes

For determination of antioxidant enzymes concentration, brain, muscle, gills, liver tissues were homogenized in 100 mmol KH_2PO_4 buffer containing 1 mmol EDTA. The homogenate was centrifuged at 12000 X g for 30 min at 4°C. The supernatant was collected and stored at -20°C until determination of antioxidant enzymes.

Catalase Assay (CAT)

The activity of CAT in the brain, gills, liver and muscle tissues of both control and CYP treated group of fishes after 24, 48, 72 and 96 hr was analyzed by following Chance and Maehly method (1955).

Briefly, 2.5 ml of 50 mM phosphate buffer (pH 5.0), 0.4 ml of 5.9 mM H_2O_2 and 0.1 ml enzyme extract were mixed. The spectrophotometer was set at 240 nm and change in absorbance of the reaction solution after one minute was recorded. The results of CAT specific activities were expressed as nmol/ min/ mg protein using molar coefficient of 43.6/ M cm.

Peroxidase assay (POD)

The activity of POD in brain, gills, liver and muscle tissue of both control and CYP treated fish after 24, 48, 72 and 96 hr was checked. Chance and Maehly method (1955), followed by Bibi (2012) was adopted. Briefly, 2.5 ml of 50 mM phosphate buffer (pH 5.0), 0.1 ml of 20 mM guaiacol, 0.3 ml of 40 mM H_2O_2 and 0.1 enzyme extract were mixed to make the reaction solution. A change in absorbance of the reaction solution at 470 nm was recorded after 1 min by spectrophotometer. POD activity was measured as nmol $min^{-1}mg^{-1}$ protein using molar coefficient of 2.66×10^4/ M cm.

Superoxide Dismutase Assay (SOD)

SOD activity was estimated by the method of Kakkar et al. (1984) with some modification. The reaction mixture contained 0.1 ml of phenazine methosulphate (186 µM), 1.2 ml of sodium pyrophosphate buffer (0.052 mM, pH 7.0), and 0.3 ml of supernatant. Enzyme reaction was initiated by adding 0.2 ml of NADH (780 µM) and stopped after 1 min by adding 1 ml of glacial acetic acid. Amount of chromogen formed was measured by recording colour intensity at 560 nm. One unit of SOD activity is defined as the amount of enzyme (per protein milligram) that inhibits the quercetin oxidation reaction by 50 percent (of maximal inhibition). Results of SOD specific activities are expressed in µmoles/ min/ mg protein using molar coefficient of 6.22×10^3/ M cm.

Glutathione Reductase Assay (GR)

The activity of GR in brain, gills, liver and muscle tissue of both control and CYP exposed fishes after 24, 48, 72 and 96 hrs was measured. Carlberg and Mannervik (1975) method was used for the determination of GR activity in the respected tissues. To prepared reaction solution ,1.65 ml phosphate buffer (0.1 M, pH 7.6), 0.1 ml EDTA (0.5 mM), 0.1 ml NADPH (0.1 mM) 0.05 ml oxidized glutathione (1 mM), and 0.1 ml of supernatant were mixed. A change in absorbance of the reaction solution at 340 nm was recorded after 1 min by spectrophotometer and GR activity was calculated as nM NADPH oxidized/ min/mg protein using molar extinction coefficient of 6.220×10^3/ M cm.

Lipid Peroxidation Assay (LPO/ TBARS)

The LPO activity in brain, gills, and liver and muscle tissues of both control and CYP treated fish after 24, 48, 72 and 96 hrs was analyzed by following the method of Wright *et al.* (1981). The 1.0 ml reaction mixtures was prepared by mixing 0.58 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml supernatant, 0.2 ml ascorbic acid (100 mM), and 0.02 ml ferric chloride (100mM). The solution was incubated in water bath for 1hr at 37°C and the reaction was stopped by addition of 1.0 ml trichloroacetic acid (10%). After addition of 1.0 ml of thiobarbituric acid, all the tubes were boiled in a water bath for 20 min and were cooled in ice bath and were centrifuged at 2500× g for 10 min. A changed in absorbance of the reaction solution at 535 nm was recorded after 1 min by spectrophotometer. The result was expressed as nM TBARS/ min/ mg tissue at 37°C using molar extinction coefficient of 1.56×10^5/ M cm.

Total Reduced Glutathione Assay (GSH)

Reduced glutathione was estimated by the method of Jollow et al. (1974). 1.0 ml sample of 10% homogenate was precipitated with 1.0 ml of (14%) sulfosalicyclic acid. The samples were kept at 4°C. The total volume of 3.0 ml assay mixture composed of 0.1 ml filtered aliquot, 2.7 ml phosphate buffer (0.1M, pH 7.4) and 0.2 ml DTNB (5,5-dithiobis-2-nitrobenzoic acid) (100mM). The yellow color of the mixture was developed, read immediately at 412 nm on a Spectrophotometer and expressed as µM GSH/g tissue.

Glutathione Peroxidase Assay (GSH-Px)

Glutathione peroxidase activity was assayed by the method of Mohandas et al. (1984). The reaction mixture consisted of 1.49 ml phosphate buffer (0.1 M, pH 7.4), 0.1 ml sodium azide (1 mM), 0.05 ml glutathione reductase (1 IU/ml), 0.05 ml GSH (1 mM), 0.1 ml EDTA (1 mM), 0.1 ml NADPH (0.2 mM), 0.01 ml H2O2 (0.25 mM) and 0.1 ml 10% homogenate in a total volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at 25°C. Enzyme activity was calculated as nM NADPH oxidized/ min/ mg protein using molar extinction coefficient of 6.22×10^3/ M cm.

Glutathione-S-Transferase Assay (GST)

Glutathione-S-transferase activity was assayed by following Habig et al. (1974). The reaction mixture consisted of 1.475 ml phosphate buffer (0.1 M, pH 6.5), 0.025 ml CDNB (2,4-Dinitrochlorobenzene, 1mM), 0.2 ml reduced glutathione (1 mM) and 0.3 ml of 10% homogenate in a total volume of 2.0 ml. the change in the absorbance was recorded at 340 nm and enzyme activity was calculated as nM CDNB conjugate formed/ min/ mg protein using a molar extinction coefficient of 9.6×10^3/ M cm.

Digestive Enzyme analysis

After 28 days of feeding, with 35% basal protein diet and Vit C in two concentration i.e., 100 mg/kg diet and 200 mg/kg diet, three fish from each aquarium were collected, anaesthetized immediately with buffered Clove oil and dissected on the ice pad and digestive tract were removed. Using hand held glass homogenizer (Model AHS 200), the intestinal contents (1 g) with 10 mL phosphate buffer (pH 7.5) was homogenized. The homogenate was then centrifuged at15000 rpm (Model Eppendorf centrifuge 5417R) for 15 minutes at 4°C. The supernatant was collected and stored at 4°C until analysis. For calculating the values of the studied digestive enzymes, protein content of the tract was also evaluated through Lowry's method (Lowry et al., 1951).

Determination of Protease Activity

Protease activity was measured by preparing 5 mL of casein solution (0.65%). The solution was prepared by dissolving 0.65 gm casein in water and incubation for 5 minutes at 37°C. Then 1 mL of enzyme solution was added and further incubated for 10 minutes at 37°C. The reaction was stopped by the addition of 5 mL of 110 mM trichloroacetic acid solution (500 ml 110 mM trichloroacetic acid solution was prepared by taking 55 ml of 1 molar stock solution and raised the volume up to 500 ml by the addition of 445 ml distilled water) and further incubated for 30 minutes at 37°C, cooled at room temperature and filtered by using Whatmann filter paper. Then 2 mL of filtered solution was taken in 10 mL test tube and added 1 mL of Folin and Ciolcaltea's reagent (0.5 mM) and 5mL of Na_2CO_3 (500 mM), incubated again at 37°C for 30 minutes. After cooling, absorbance was measured at 660 nm with the help of UV-Visible spectrophotometer.

Determination of Amylase Activity

Amylase activity was evaluated by 3, 5-Dinitrosalicylic acid (DNS) method (Bernfeld, 1955) adopted by Areekijseree *et al.* (2004) with a few modifications. The method was based on the estimation of reducing sugars at 560 nm using maltose as the standard. About 0.5 mL of enzyme solution was incubated for 3-4 minutes at room temperature. Then 500 µL of 1% starch (1 g starch / 100 mL distilled water) solution was added and kept at ambient temperature for 3 minutes, followed by the addition of 1 mL of DNS reagent (100 ml DNS reagent was prepared by dissolving 18.2g Na K Tartarate, 1g NaOH, 1 g DNS, 0.2 g phenol and 0.05 g Na_2SO_4 in 100 ml distilled water) and incubated on boiling water bath for about 5 minutes. After cooling at room temperature 10 mL of reagent grade water was added and absorbance was noted at 540 nm on spectrophotometer.

One amylase unit was defined as the amount of enzyme/ mL filtrate that released one microgram reducing sugar/ minute.

Determination of Cellulase Activity

The activity of digestive enzyme, cellulase was measured by following the methodology of Denison and Kohen (1977) with few modifications. Briefly, citrate phosphate buffer of pH 5 was prepared by mixing 0.3708 g citric acid and 2.932 g of sodium phosphate in 100 mL H_2O, with addition of 1% carboxymethyl cellulase solution (1 g CMC/ 100 mL of H_2O). Then reaction mixture containing 1 mL of appropriate enzyme solution in test tube, 1 mL of CMC solution and 1mL of citrate buffer (0.1 M) was prepared and incubated at 50°C for about half an hr.

After incubation, test tubes with 3 mL DNS reagent were boiled in water bath for 15 minutes. Then 1 mL of 40% sodium potassium tartarate was added in test tubes and allowed to cool at room temperature. After cooling, the production of reducing sugar (glucose) from CMC substrate resulted by cellulolytic activity was measured at 540 nm on UV- Visible spectrophotometer.

One unit cellulase activity is defined as the amount of enzyme/ mL culture filtrate that released 1 mg glucose/ minutes.

Enzymes of Nitrogen Metabolism/ Protein Metabolism

Aspartate Aminotransferase (AST)

The activity of aspartate aminotransferase (AST) was assayed by the method described by Bergmeyer (1965). 2 % w/v tissue homogenates of the selected tissues were prepared in 0.25 M ice cold sucrose solution. The homogenates were centrifuged at 1000xg for 15 minutes and supernatant was used for the enzyme assay. The incubation mixture of 2.0 ml contained 100 µ moles of phosphate buffer (Na_2HPO_4 + NaH_2PO_4) (pH 7.4), 100µmoles of L-aspartatic acid, 2 µ moles of α-keto glutarate and 0.5 ml of supernatant as enzyme source. After incubation for 30 minutes at 37°C, the reaction was stopped by the addition of 1 ml of ketone reagent (0.001 M, 2,4-dinitrophenyl hydrazine solution in 1 N HCl) and the contents were allowed to stay at laboratory temperature for 20 minutes. After 20 minutes of 10 ml of 0.4 N NaOH was added. The developed colour was read at 545 nm in a spectrophotometer against a reagent blank.

Alanine Aminotransferase (ALT)

The activity of alanine aminotransferase (ALT) was assayed by the method of described by Bergmeyer (1965). The incubation mixture of 2 ml contained 100 µ moles of DL-alanine, 100 µ moles of phosphate buffer (pH 7.4), 2 µ moles of α-ketoglutarate and 0.5 ml of the supernatant of the homogenate 2% w/v prepared in 0.25 M ice-cold sucrose solution, as enzyme source. The reaction mixture was incubated at 37°C for 30 minutes. The reaction was stopped by the addition of 1.0 ml of 2, 4-dinitrophenyl hydrazine solution prepared in 1 N HCl (ketone reagent). The colour was developed by the addition of NaOH as described above for AST. The optical density was measured at 545 nm in a spectrophotometer against a reagent blank.

Glutamate Dehydrogenase (GDH)

Glutamate dehydrogenase (GDH) was assayed using the method of Lee and Hardy (1965) and expressed as µM formozan/mg protein/hr. Glutamate dehydrogenase activity was assayed in a 2 ml volume containing phosphate buffer (100 µmol, pH 7.4) sodium glutamate (75 µmol), INT (2,4-iodophenyl-3-(4-nitrophenyl)-5-phenyltetrazolium chloride, 2 µmol) NAD (0.1 µmol) and enzyme source (0.5 ml) prepared in sucrose solution (0.25 M). The 2ml

volume of reaction mixture was incubated for 30 min. Measurements of enzyme activities were performed spectrophotometrically at 340 nm.

Lactate Dehydrogenase (LDH)

The activity of this enzyme (μM Pyruvate/ mg protein/ hr) was determined by the formation of NADH by following Franciscato et al. (2011). The medium containing buffer 200 mmol/L pH 8.2, lactic acid 260 mmol/L, sodium azide 7.7 mmol/ L and 25 mL of plasma was incubated at 37 1C for 2 min. After this period, the colour reactive (INT 0.64 mmol/L, NAD+ 1.2 mmol/L, phenazine 0.26 mmol/L and sodium azide 1.23 mmol/L) was added and the medium was incubated for another 5 min at 37°C. The reaction was stopped by adding HCl 200 mmol/L and the tubes remained at room temperature for 5 min until the reading of the absorbance at 500 nm.

Determination/Estimation/Computation/Assessment of DNA damage

DNA damage of was evaluated by using a modified neutral single cell electrophoresis (SCGE / comet assay) according to the method of Singh et al. (1988) as reviewed by Lee and Steinert (2003). The comet assay (SCGE) is a simple practice used for noticing single and double strand DNA breaks and alkali-labile sites in DNA.

Single Cell Gel Electrophoresis (SCGE) / Comet assay

This practice includes, embedding of single cell in agarose, lysing the cells and liberating out DNA by electrophoresis to form a comet tail cleaving the head of intact DNA. Intact and damaged DNA is pictured by fluorescence microscopy and computed by image analysis. Following are the basic steps of comet assay:

- Microscope slides preparation layered with cells in agarose
- Lysis of membranes to release DNA
- Single strand DNA formed by contact to alkali (pH-13)
- Neutral Electrophoresis
- DNA staining and analysis
- Comet scoring

The whole procedure was performed under low light in order to avoid induced damage to DNA. The detail of each step is as follows.

Slide Preparation

Frosted microscopic slides were moderately heated on slide warmer, sheltered with 100µL of 1% regular melting point agarose (RMPA) prepared in distilled water at 40°C and straightaway covered with a large (22 x 50mm) coverslip. The slides were kept in a chilled tray and left at 4°C for at least 30 min to let the agarose to solidify. The coverslips were then removed and second layer of 85 µL was made on top of the first layer containing 20 µL of blood suspension and 65µL of 1% low melting point agarose (LMPA) at 37°C. This cell suspension creates a layer on top of the first agarose layer, covered with a coverslip and permitted to solidify.

Lysis

The cells were then lysed by eliminating the coverslips and immersing the slides in a histology jar containing freshly made cold lysis buffer (pH =10.3). The lysis buffer had 2.5 M NaCl, 100 mM EDTA, 10 mM Tris Base, (pH= 10.3), 1% (w/v) Triton X-100 (Dithiothreitol). Triton X-100 was added just afore commencing the lysis. EDTA was dissolve with the aid of NaOH pellets @ 0.2 g/ 10ml of solution. The slides were incubated with lysing solution for 24 hr at room temperature. After the incubation the slides were sweep away with distilled water three times at 20 min pauses by transferring the rack from one jar to another, to remove traces of salt and detergent. The jar was topped with aluminium foil to minimize light.

Neutral Electrophoresis

Slides were steadily placed in columns in the electrophoresis tray fronting towards anode containing a mixture of 1200 ml distilled water and 300 ml neutral electrophoresis buffer (54 g/L Tris base, 27.5 g/L boric acid, 0.5 M EDTA) at pH=8.0. Boric acid was dissolved at 45 °C while stirring. Slides were then equilibrated with electrophoresis buffer for 20 min. Electrophoresis was accomplished for 20 min at 25V (0.714 V/ cm).When electrophoresis was finished, the buffer was drained from the tank and the tray removed. Slides were covered with aluminium foil and air-dried overnight at 5°C.

Analysis and Scoring of the Slides

The slides were rehydrated with distilled water for 60 min stained with Acridine orange (300-400 µL of 20µg/ml of distilled water) and examined under epifluorescent microscope (400X, Nikon AFX-1 Optiphot) Digital images were taken for succeeding analyses/scoring with TRITEK software. For analysis, 120 cells were counted from four fields of each slide counting the intact DNAs and the number of comets. DNA comet parameters including Comet length (CL, µm), Head length (HD, µm), % DNA in head (%H),

Tail length (TL, μm), Tail DNA (TDNA, %), Tail moment (TM, μm) and Olive moment (OTM, μm) were recorded.

Statistical Analysis

Data obtained from experiment were expressed as mean±SE. The result was analyzed by using one way analysis of variances (ANOVA) followed by LSD test using Statistix Version 8.1. Values of $P < 0.05$ were considered statistically significant.

RESULTS

RESULTS

Toxicity Evaluation

An increase was observed in mortality with elevation in CYP concentration, shown in Table 1. Plotting percent mortalities against CYP log concentration resulted in a curve, showing log concentration number 4 (4.5 µg/L) that caused 50% mortality after 96 hr of exposure, shown in Fig 1. To clarify 96 hr LC_{50}, for CYP against rohu, Probit Analysis was carried out by plotting Probit mortality against CYP log concentration. This showed 50% mortality after 96 hr at Log concentration 0.653 (equal to 4.5 µg/L), shown in Fig 2. Further statistical verification was done through Dragstedt-Behren's equation resulted in a value of 4.49µg/L. The LC_{50} (4.497 µg/L, almost equal to 4.5 µg/L) was selected after taking the average of the above three methods, shown in Table 2.

Behavioural Study

Fish exposed to acute concentration of CYP showed different time relying behavioural changes (Table 3). During first 24 hr of exposure fish showed jumping, erratic swimming, hyper activeness, hyperventilation, aggression and equilibrium loss in fish of Group 2nd (fish fed basal diet) and Group 3rd (fish fed vitamin C supplemented diet at the rate of 100 mg/Kg diet) while fish in Group 4th (fish fed vitamin enriched diet at the rate of 200 mg/kg diet) only showed hyper activeness. Rest of the changes was not observed in Group 4th and these changes were completely absent in fish of Group 1st (Control, fed basal diet and did not exposed to CYP). Till 48 hr fish in Group 2nd and Group 3rd showed hyper activeness while then onward showed hypo activeness and increased air gulping. After 48-96 hr of the exposure the fish showed reflex loss, motionless state and adopting vertical position in Group 2nd whereas these changes was not observed in fish of Group 1st, Group 3rd and Group 4th. Fish in Group 2nd showed aggregation at aquaria corner vertically after 72-96 hr of exposure to CYP. Fish in Group 1st did not showed any of these changes whereas fish from Group 4th only showed hyper activeness initially, which was not observed after a few hr of exposure. Table 3 is showing different behavioural responses shown by *Labeo rohita* in different groups of fish.

Tissues' Biochemical Studies

Protein Estimation

Total protein content observed in various tissues of different groups of fish at various time intervals is presented in Fig 3. In brain, gills, liver and muscle tissues, CYP exposure caused highest time dependent gradual decrease in protein content in a group of fish fed basal diet (Group 2nd), then followed by a group of fish fed diet supplemented with vitamin C at the rate of 100 mg/kg diet (Group 3rd). However, the total protein content in different tissues of fish in Group 1st (Control), not exposed to CYP, and Group 4th fed vitamin C enriched diet (200 mg/Kg diet) before exposure to CYP was considerably similar but higher than observed in Group 2nd and Group 3rd. Tables 4 to 7 are respectively showing total protein content in brain, gills, liver and muscle tissues of rohu after exposure to acute concentration of CYP.

Lipid Peroxidation Assay (LPO/ TBARS)

Lipid peroxidation (LPO) level observed in various tissues of different groups of fish at various time intervals after CYP exposure is presented in Fig 4. In brain, gills, liver and muscle tissues, CYP exposure caused highest time dependent increase in LPO level in a group of fish fed basal diet (Group 2nd) followed by fish fed diet supplemented with vitamin C at the rate of 100 mg/Kg diet (Group 3rd). However, LPO in different tissues in Group 1st (Control, not exposed to CYP) and Group 4th fed vitamin C enriched diet (200 mg/Kg diet) was similar but higher than observed LPO in Group 2nd and Group 3rd. Tables 8 to 11 are showing LPO activity in brain, gills, liver and muscle tissues of rohu.

Catalase Assay (CAT)

Catalase (CAT) activity observed in different tissues of different groups of fish at various time intervals is presented in Fig 5. Exposure of acute concentration of CYP caused highest time dependent increase in catalase activity in fish fed basal diet (Group 2nd) followed by fish fed diet supplemented with vitamin C at the rate of 100 mg/Kg diet (Group 3rd). However CAT activity in different tissues of fish in Group 1st (Control, not exposed to CYP) and Group 4th fed vitamin C enriched diet (200 mg/Kg diet) before exposure to CYP was considerably similar but higher than observed in Group 2nd and Group 3rd. Tables 12 to 15 are showing CAT activity in brain, gills, liver and muscle tissues of different groups of fish.

Peroxidase assay (POD)

Peroxidase (POD) activity observed in different tissues of different groups of fish at various time intervals is presented in Fig 6. Exposure of acute concentration of CYP caused highest time dependent increase in catalase activity in fish fed basal diet (Group 2nd) followed by fish fed diet supplemented with vitamin C at the rate of 100 mg/Kg diet (Group 3rd). However CAT activity in different tissues of fish in Group 1st (Control, not exposed to CYP) and Group 4th fed vitamin C enriched diet (200 mg/Kg diet) before exposure to CYP was considerably similar but higher than observed in Group 2nd and Group 3rd. Tables 16 to 19 are showing CAT activity in brain, gills, liver and muscle tissues of different groups of fish.

Superoxide Dismutase Assay (SOD)

Superoxide dismutase activity was observed to be on increasing trend in different tissues of fish fed with basal diet (Group 2nd) followed by fish fed diet supplemented with vitamin C at the rate of 100 mg/kg diet (Group 3rd), shown in Fig 7. SOD activity in Group 1st (Control, not exposed to CYP) and Group 4th fed vitamin C enrich diet (200 mg/Kg diet) before exposure to CYP was considerably similar but higher than observed in Group 2nd and Group 3rd. Tables 20 to 23 are showing SOD activity in brain, gills, liver and muscle tissue of different groups of fish.

Glutathione Reductase Assay (GR)

Activity of glutathione reductase observed in different tissue of different groups of fish at different time intervals is shown in Fig 8. Highest GR activity was observed in fish fed basal diet (Group 2nd) followed by fish fed diet supplemented with vitamin C at the rate of 100 mg/Kg diet (Group 3rd). GR activity in studied tissues was observed to be considerably similar in Group 1st (Control, not exposed to CYP) and fish fed diet supplemented with vitamin C at the rate of 200 mg/Kg diet before exposure to CYP. Tables 24 to 27 are showing GR activity in brain, gills, liver and muscles tissues of different groups of fish at various time interval.

Total Reduced Glutathione Assay (GSH)

Total reduced glutathione observed in different tissues of different groups at different time intervals is presented in Fig 9. A highest increasing time dependent trend in GSH activity was observed in Group 2nd (Fish fed basal diet) followed by Group 3rd (fish fed diet supplemented with vitamin C at 100 mg/Kg diet) while no considerable difference was observed between Group 1st (Control) and Group 4th (fish fed diet supplemented with vitamin

C at the rate of 200 mg/Kg diet) in GSH activity. Tables 28 to 31 are showing GSH activity in brain, gills, liver and muscles of different groups of fish.

Glutathione Peroxidase Assay (GSH-Px)

Glutathione peroxidase activity observed in different tissues of different groups at different time intervals is shown in Fig 10. In brain, gills, liver and muscle tissues, CYP exposure caused highest time dependent gradual increase in GSH-Px activity in a group of fish fed basal diet (Group 2nd), followed by a group of fish fed diet supplemented with vitamin C at the rate of 100 mg/Kg diet (Group 3rd). However, the GSH-Px activity in different tissues of fish in Group 1st (Control, not exposed to CYP) and Group 4th fed vitamin C enrich diet (200 mg/Kg diet) before exposure to CYP was considerably similar but higher than observed in Group 2nd and Group 3rd. Tables 32 to 35 are showing GSH-Px activity in brain, gills, liver and muscle tissues of different groups of fish.

Glutathione-S-Transferase Assay (GST)

Glutathione-S-transferase activity observed in brain, gills, liver and muscles of rohu at different time intervals is given in Fig 11. Exposure to CYP increased glutathione transferase (GST) activity in all four studied tissue in a group fed with basal diet (Group 2nd) followed by a group fed diet supplemented with vitamin C at the rate of 100 mg/Kg diet (Group 3rd). No considerable change was observed between Group 1st (Control, not exposed to CYP) and fish fed vitamin C enrich diet at the rate of 200 mg/Kg diet (Group 4th). Tables 36 to 39 are presenting GST activity in brain, gills, liver and muscle of rohu in different groups of fish at different times intervals.

Digestive Enzymes

Exposure of CYP elevated the activities of digestive enzymes including Amylase, Cellulase and Protease in rohu, *Labeo rohita*. A statistically significant difference ($P < 0.05$) was observed in Group 1st, Group 2nd and Group 3rd while no significant difference was observed between Group 1st and Group 4th except at 24 and 48 hr in Amylase and Protease activities. Activities of digestive enzymes observed in different groups of fish at different time intervals are given in Fig 12.

Amylase Activity

Amylase activity (U/mg) was observed to be 0.219±0.03 and 0.215±0.02 in Group 1st, 0.377±0.06 and 0.481±0.04 in Group 2nd, 0.266±0.01 and 0.328±0.01 in Group 3rd, and

0.212±0.021 and 0.213±0.047 in Group 4th at 24 and 96 hr respectively. Table 40 is showing Amylase activity in rohu in different groups of fish at different time intervals.

Cellulase Activity

Cellulase activity (U/mg) was observed to be 0.788±0.052 and 0.789±0.052 in Group 1st, 0.947±0.116 and 1.140±0.114 in Group 2nd, 0.859±0.018 and 0.947±0.031 in Group 3rd, and 0.787±0.038 and 0.786±0.023 in Group 4th at 24 and 96 hr of CYP exposure respectively. Table 41 is showing Cellulase activity in different groups of rohu at 96 hr.

Protease Activity

Protease activity (U/mg) was observed to be 0.052±0.006 and 0.053±0.023 in Group 1st, 0.101±0.064 and 0.195±0.041 in Group 2nd, 0.097±0.003 and 0.124±0.003 in Group 3rd, and 0.054±0.007 and 0.054±0.006 in Group 4th at 24 and 96 hr respectively. Table 42 is showing Protease activity in different groups of rohu at 96 hr.

Enzymes of Nitrogen Metabolism/ Protein Metabolism

CYP exposure caused highest increase in enzymes of protein metabolism in a group of fish fed basal diet (Group 2nd), followed by fish fed diet supplemented with vitamin C at the rate of 100 mg/Kg diet (Group 3rd). The activities of enzymes of nitrogen metabolism in Fish of Group 1st (Control, not exposed to CYP) was considerably similar to fish fed enrich vitamin C supplemented diet, 200 mg/Kg diet (Group 4th) in activities of enzymes of nitrogen metabolism. Fig 13 to 16 is showing AST, ALT, GDH and LDH activities in different group of fish.

Aspartate Aminotransferase (AST)

AST activity observed in liver, muscle and gills of different group of fish are presented in Tables 43 to 45. AST activity showed highest time dependent increasing trend in fish fed basal diet (Group 2nd) followed by fish fed diet supplemented with vitamin C at the rate of 100 mg/kg diet (Group 3rd). AST activity in fish of Group 1st (Control) and fish of Group 4th fed vitamin C enrich diet (200 mg/Kg diet) before exposure to CYP was considerably similar.

Alanine Aminotransferase (ALT)

ALT activity observed in liver, muscles and gills of different groups of fish are presented in Tables 46 to 48 respectively. ALT activity was observed to be on highest time dependent increasing trend in fish fed basal diet (Group 2nd) followed by fish fed diet supplemented with vitamin C at the rate of 100 mg/kg diet (Group 3rd). ALT activity in fish of Group 1st (Control) and fish of Group 4th fed vitamin C enriched diet (200 mg/Kg diet) was considerably similar.

Glutamate Dehydrogenase (GDH)

GDH activity observed in gills, liver and muscles tissues of different groups of fish are given in Tables 49 to 51. GDH activity at different time intervals showed similar trend as observed in AST and ALT in all the groups of fish.

Lactate Dehydrogenase (LDH)

LDH activity observed in liver, muscles and gills of different groups of fish are given in Tables 52 to 54. LDH activity was observed, statistically highest in fish fed basal diet (Group 2nd) followed by fish fed vitamin C supplemented diet at the rate of 100 mg/Kg diet (Group 3rd). LDH activity in fish of Group 1st (Control, not exposed to CYP) and fish of Group 4th fed enriched vitamin C diet (200 mg/Kg diet) was considerably similar.

Evaluation of DNA damage through Comet Assay

DNA damage was evaluated by Comet Assay from the peripheral blood erythrocyte of rohu, *Labeo rohita*. The damage was time dependent. Table 55 to 58 are showing DNA damage in different groups of fish at 24, 48, 72 and 96 hr respectively. Fig 25 is showing DNA damage observed in Group 2nd and Group 3rd while intact DNA in Group 1st and Group 4th.

Observed number of comets/120 cells, comet lengths, tail length, head length, %Head DNA, %Tail DNA, Tail movement and Olive tail movement in all groups are given in Fig 17 to 24 respectively.

Table 1: Determination of LC_{50} value of CYP for 96 h r

S. No	Concentration CYP (µg/L)	Log Concentration	Number of fish exposed	No. of alive fish	No. of dead fish	Percent mortality (%)	Probit Mortality
1	3.0	0.477121255	10	10	0	0	0.00
2	3.5	0.544068044	10	9	1	10	3.72
3	4.0	0.602059991	10	7	3	30	4.48
4	4.5	0.653212514	10	5	5	50	5.00
5	5.0	0.698970004	10	3	7	70	5.52
6	5.5	0.740362689	10	1	9	90	6.28
7	6.0	0.778151250	10	0	10	100	8.09

Table 2: Average LC_{50} value of CYP for *Labeo rohita*

S. No	Method Name	LC_{50} Value in µg/L
1	Percent Mortality	4.5 µg/L
2	Probit Morality	4.5 µg/L
3	Dragstedt-Behren's Method/Equation	4.49 µg/L
	Mean	4.497 µg/L
	Standard Deviation	0.0058
	Standard Error	0.0033

Table 3: Behavioral response of *L. rohita* exposed to LC_{50} CYP

Behavior	Treatment groups			
	Control	CYP	CYP+Vit C_{100}	CYP+Vit C_{200}
24 hr				
Jumping	–	+	–	–
Erratic swimming	–	+	+	–
Equilibrium Loss	–	+	+	–
Air gulfing	–	+	+	–
Hyperventilation	–	+	+	–
Hyper activeness	–	+	+	+
48-96 hr				
Hypo activeness	–	+	+	–
Reflex loss	–	+	–	–
Discoloration	–	+	+	–
Vertical position	–	+	–	–
Motionless state	–	+	–	–

+ = Present; – = Absent

Table 4: Total Protein Content (mg/ g) in brain of different groups of fish at various time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	9.82 ± 0.53^{a}	9.12 ± 0.38^{e}	9.55 ± 0.26^{c}	9.72 ± 0.23^{ab}
48	9.69 ± 0.56^{abc}	8.42 ± 0.64^{f}	9.22 ± 0.48^{d}	9.65 ± 0.36^{abc}
72	9.85 ± 0.36^{a}	8.12 ± 0.44^{g}	8.65 ± 0.48^{f}	9.82 ± 0.19^{a}
96	9.69 ± 0.56^{abc}	7.65 ± 0.48^{h}	7.95 ± 0.43^{g}	9.63 ± 0.76^{abc}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 5: Total Protein Content (mg/ g) in gills of different groups of fish at various time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	7.69 ± 0.56^{b}	7.25 ± 0.45^{e}	7.42 ± 0.42^{d}	7.68 ± 0.38^{b}
48	7.82 ± 0.69^{a}	6.82 ± 0.38^{h}	7.02 ± 0.48^{f}	7.85 ± 0.37^{a}
72	7.65 ± 0.57^{c}	6.12 ± 0.45^{j}	6.92 ± 0.45^{g}	7.59 ± 0.35^{c}
96	7.75 ± 0.52^{b}	5.82 ± 0.48^{k}	6.45 ± 0.33^{i}	7.69 ± 0.29^{b}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 6: Total Protein Content (mg/ g) in liver of different groups of fish at various time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	16.092 ± 0.13^{d}	13.157 ± 0.73^{i}	15.558 ± 0.69^{e}	16.025 ± 0.19^{d}
48	16.191 ± 0.50^{d}	13.123 ± 0.44^{j}	14.957 ± 0.39^{f}	15.891 ± 0.39^{d}
72	16.958 ± 0.24^{a}	10.445 ± 0.57^{k}	13.457 ± 0.46^{h}	16.225 ± 0.25^{c}
96	16.858 ± 0.11^{b}	9.755 ± 0.65^{l}	13.824 ± 0.60^{g}	16.891 ± 0.65^{b}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 7: Total Protein Content (mg/ g) in muscles tissues of different groups of fish at various time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	12.490 ± 0.48^{d}	11.689 ± 0.48^{h}	11.989 ± 0.40^{f}	12.423 ± 0.53^{d}
48	12.423 ± 0.39^{d}	10.889 ± 0.34^{i}	11.789 ± 0.50^{g}	12.456 ± 0.45^{d}
72	12.723 ± 0.28^{c}	10.188 ± 0.55^{j}	10.889 ± 0.48^{i}	12.923 ± 0.28^{a}
96	12.756 ± 0.61^{b}	9.195 ± 0.39^{l}	10.022 ± 0.58^{k}	12.089 ± 0.29^{e}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 8: Activity of LPO (µmol/ min/ mg protein) in Brain of different groups of fish at various time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	16.411 ± 0.877^{h}	29.411 ± 1.198^{de}	22.267 ± 0.402^{f}	16.396 ± 0.607^{h}
48	16.724 ± 0.886^{g}	39.596 ± 2.709^{c}	27.196 ± 1.465^{e}	16.689 ± 0.84^{g}
72	16.731 ± 0.476^{g}	49.125 ± 2.329^{b}	32.848 ± 1.759^{d}	16.695 ± 0.299^{g}
96	16.709 ± 0.801^{g}	63.188 ± 3.712^{a}	35.299 ± 1.810^{cd}	16.689 ± 1.309^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 9: Activity of LPO (µmol/ min/ mg protein) in Gills of different groups of fish at various time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	11.102 ± 0.741^{h}	16.246 ± 1.272^{f}	12.076 ± 0.698^{g}	10.997 ± 0.326^{h}
48	11.684 ± 0.844^{h}	23.387 ± 1.201^{d}	19.051 ± 1.109^{e}	11.677 ± 0.291^{h}
72	11.813 ± 0.706^{h}	29.536 ± 1.949^{b}	25.962 ± 1.801^{c}	11.781 ± 0.681^{h}
96	11.643 ± 0.654^{h}	36.091 ± 2.753^{a}	28.091 ± 1.308^{bc}	11.666 ± 0.499^{h}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 10: Activity of LPO (µmol/ min/ mg protein) in Liver of different groups of fish at various time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	18.745 ± 0.168^{g}	32.104 ± 1.714^{e}	19.533 ± 0.211^{g}	18.837 ± 0.211^{g}
48	18.788 ± 0.494^{g}	46.351 ± 1.608^{c}	26.823 ± 0.557^{f}	18.646 ± 0.452^{g}
72	18.322 ± 0.287^{g}	69.173 ± 3.409^{b}	32.462 ± 1.071^{g}	18.313 ± 0.321^{g}
96	18.729 ± 0.021^{g}	90.037 ± 5.858^{a}	40.362 ± 1.808^{d}	18.715 ± 0.781^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 11: Activity of LPO (µmol/ min/ mg protein) in Muscles of different groups of fish at various time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	16.533 ± 0.542^{h}	22.409 ± 0.689^{f}	19.542 ± 0.299^{g}	16.543 ± 0.692^{h}
48	16.917 ± 0.486^{h}	31.794 ± 0.908^{d}	23.017 ± 1.109^{f}	16.849 ± 0.568^{h}
72	16.661 ± 0.329^{h}	41.206 ± 2.299^{b}	26.491 ± 1.107^{e}	16.597 ± 0.427^{h}
96	16.697 ± 0.667^{h}	45.873 ± 1.825^{a}	33.276 ± 1.814^{c}	16.703 ± 0.441^{h}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 12: Activity of Catalase (µmol/ min/ mg protein) in Brain of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	128.931±20.53^{g}	138.879±12.247^{de}	130.968±4.499^{f}	128.989±1.395^{g}
48	129.103±6.32^{g}	146.418±12.729^{c}	133.685±6.628^{ef}	128.985±3.824^{g}
72	128.498±11.52^{g}	156.866±8.078^{b}	137.725±10.284^{e}	128.273±2.309^{g}
96	128.125±7.34^{g}	161.816±9.997^{a}	141.082±7.869^{d}	128.054±9.363^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different (P < 0.05). (ANOVA followed by HSD Tukey test).

Table 13: Activity of Catalase (µmol/ min/ mg protein) in Gills of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	173.079±12.101^{f}	185.357±8.391^{d}	177.147±7.114^{e}	172.926±11.085^{f}
48	172.896±13.623^{f}	192.762±9.999^{c}	184.213±8.778^{d}	172.228±14.135^{f}
72	172.449±18.908^{f}	198.727±14.312^{b}	186.873±11.724^{d}	172.545±12.188^{f}
96	172.708±11.068^{f}	205.535±10.996^{a}	192.205±5.538^{c}	172.541±6.410^{f}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different (P < 0.05). (ANOVA followed by HSD Tukey test).

Table 14: Activity of Catalase (µmol/ min/ mg protein) in Liver of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	244.936±12.101^{g}	263.746±13.901^{d}	250.861±19.999^{f}	243.841±12.161^{g}
48	244.509±19.249^{g}	273.815±15.052^{c}	258.586±16.752^{e}	244.009±14.912^{g}
72	244.893±13.471^{g}	292.728±14.648^{b}	265.167±16.297^{d}	244.551±14.6554^{g}
96	245.111±12.066^{g}	304.755±20.019^{a}	272.307±11.847^{c}	244.890±10.339^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different (P < 0.05). (ANOVA followed by HSD Tukey test).

Table 15: Activity of Catalase (µmol/ min/ mg protein) in Muscles of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	222.061±7.906^{g}	233.314±8.032^{e}	229.855±7.519^{f}	222.729±9.645^{g}
48	222.727±7.072^{g}	248.869±8.230^{c}	234.032±9.747^{e}	223.114±8.719^{g}
72	223.455±5.360^{g}	266.599±11.454^{b}	239.899±11.892^{de}	223.182±9.809^{g}
96	223.105±12.574^{g}	275.045±10.454^{a}	245.443±14.367^{d}	223.622±4.421^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different (P < 0.05). (ANOVA followed by HSD Tukey test).

Table 16: Activity of POD (µmol/ min/ mg protein) in Brain of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	43.943 ± 5.374^{h}	63.945 ± 4.089^{e}	47.454 ± 2.014^{g}	44.325 ± 1.808^{h}
48	44.694 ± 4.218^{h}	80.249 ± 9.359^{c}	53.267 ± 4.577^{f}	44.715 ± 2.965^{h}
72	45.022 ± 2.679^{h}	98.452 ± 6.991^{b}	65.289 ± 5.736^{de}	44.929 ± 2.257^{h}
96	45.086 ± 3.996^{h}	114.894 ± 8.522^{a}	67.718 ± 6.156^{d}	44.925 ± 5.397^{h}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 17: Activity of POD (µmol min/ mg protein) in Gills of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	70.815 ± 7.685^{h}	122.757 ± 12.487^{d}	76.101 ± 6.759^{g}	70.944 ± 5.299^{h}
48	71.043 ± 3.796^{h}	162.187 ± 18.036^{c}	80.791 ± 5.358^{f}	71.044 ± 11.486^{h}
72	72.132 ± 7.809^{h}	212.672 ± 24.172^{b}	85.321 ± 10.301^{ef}	71.982 ± 5.940^{h}
96	72.152 ± 8.042^{h}	258.733 ± 45.925^{a}	89.016 ± 8.636^{e}	72.245 ± 4.745^{h}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 18: Activity of POD (µmol/ min/ mg protein) in Liver of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	56.184 ± 0.872^{i}	93.948 ± 6.218^{d}	62.422 ± 4.544^{h}	55.959 ± 2.557^{i}
48	57.789 ± 2.696^{i}	128.918 ± 13.7001^{c}	71.861 ± 4.479^{g}	56.579 ± 1.759^{i}
72	57.789 ± 2.723^{i}	148.645 ± 32.042^{b}	81.535 ± 7.618^{f}	57.655 ± 3.364^{i}
96	57.337 ± 0.645^{i}	183.632 ± 3.659^{a}	88.472 ± 14.698^{e}	57.650 ± 3.801^{i}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 19: Activity of POD (µmol/ min/ mg protein) in Muscles of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	28.528 ± 1.191^{f}	36.153 ± 3.154^{d}	30.847 ± 7.909^{e}	28.454 ± 9.249^{f}
48	29.009 ± 10.511^{f}	43.672 ± 9.884^{c}	33.495 ± 12.333^{de}	28.485 ± 10.713^{f}
72	28.497 ± 1.058^{f}	53.445 ± 4.033^{b}	37.343 ± 8.197^{d}	28.412 ± 9.015^{f}
96	28.638 ± 2.118^{f}	67.097 ± 5.406^{a}	48.929 ± 6.308^{bc}	28.516 ± 9.314^{f}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 20: Activity of SOD (µmol/ min/ mg protein) in Brain of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	125.006 ± 7.277^{g}	145.590 ± 5.211^{d}	132.525 ± 3.817^{f}	124.974 ± 2.834^{g}
48	125.665 ± 7.453^{g}	156.833 ± 9.849^{c}	137.797 ± 7.185^{e}	125.791 ± 4.469^{g}
72	125.315 ± 4.777^{g}	167.128 ± 8.496^{b}	142.536 ± 7.405^{de}	125.109 ± 2.809^{g}
96	126.171 ± 7.266^{g}	178.173 ± 10.998^{a}	149.797 ± 7.479^{cd}	125.816 ± 9.714^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 21: Activity of SOD (µmol/ min/ mg protein) in Gills of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	143.996 ± 4.537^{h}	165.872 ± 9.201^{f}	153.789 ± 8.808^{g}	143.949 ± 6.867^{h}
48	143.159 ± 12.836^{h}	196.309 ± 0.228^{c}	167.083 ± 10.099^{ef}	143.343 ± 7.287^{h}
72	144.226 ± 9.927^{h}	199.771 ± 14.283^{b}	168.538 ± 8.888^{e}	144.178 ± 6.798^{h}
96	144.233 ± 7.664^{h}	221.269 ± 17.742^{a}	181.517 ± 8.769^{d}	144.199 ± 8.227^{h}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 22: Activity of SOD (µmol/ min/ mg protein) in Liver at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	162.369 ± 4.888^{g}	173.299 ± 9.033^{de}	169.993 ± 6.035^{f}	162.462 ± 2.197^{g}
48	162.011 ± 3.456^{g}	184.094 ± 6.671^{c}	172.461 ± 6.517^{e}	162.517 ± 3.979^{g}
72	162.876 ± 2.988^{g}	198.169 ± 9.373^{b}	174.115 ± 5.129^{d}	162.903 ± 2.858^{g}
96	163.067 ± 0.605^{g}	208.041 ± 14.298^{a}	179.028 ± 6.049^{cd}	162.959 ± 7.974^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 23: Activity of SOD (µmol/ min/ mg protein) in Muscles at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	136.891 ± 3.836^{h}	147.558 ± 7.701^{f}	141.178 ± 8.635^{g}	136.250 ± 9.718^{h}
48	137.014 ± 6.397^{h}	167.308 ± 4.961^{c}	149.248 ± 5.363^{f}	136.466 ± 5.455^{h}
72	136.686 ± 3.724^{h}	174.341 ± 10.947^{b}	158.777 ± 7.273^{e}	136.619 ± 4.319^{h}
96	136.264 ± 7.085^{h}	193.494 ± 9.787^{a}	162.977 ± 8.679^{d}	136.092 ± 3.197^{h}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 24: Activity of GR (µmol NADPH oxidized/ min/ mg protein) in Brain of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1216.993±64.182^{i}	1702.538±41.514^{d}	1443.798±23.005^{g}	1218.277±20.734^{i}
48	1220.082±67.216^{i}	2160.344±159.492^{c}	1496.672±71.130^{f}	1218.984±21.288^{i}
72	1226.459±40.508^{h}	2471.882±113.918^{b}	1593.266±87.261^{e}	1227.029±23.739^{h}
96	1227.505±67.102^{h}	2982.9001±183.865^{a}	1699.581±90.545^{d}	1226.249±92.965^{h}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different (P < 0.05). (ANOVA followed by HSD Tukey test).

Table 25: Activity of GR (µmol NADPH oxidized/ min/ mg protein) in Gills of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	421.254±28.488^{g}	492.111±25.161^{e}	482.693±48.418^{f}	421.322±28.462^{g}
48	424.992±34.572^{g}	523.111±61.518^{d}	493.174±55.915^{e}	421.673±7.726^{g}
72	423.355±24.487^{g}	613.504±62.579^{b}	529.683±36.655^{cd}	421.565±58.822^{g}
96	424.901±37.291^{g}	695.154±41.396^{a}	531.671±23.248^{c}	424.019±6.946^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different (P < 0.05). (ANOVA followed by HSD Tukey test).

Table 26: Activity of GR (µmol NADPH oxidized/ min/ mg protein) in Liver of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1430.915±40.758^{g}	1694.264±87.026^{f}	1513.934±64.769^{g}	1432.505±18.759^{g}
48	1430.758±46.404^{g}	2181.937±80.778^{d}	1825.135±47.999^{e}	1432.172±29.464^{g}
72	1439.004±20.402^{g}	2865.734±150.734^{b}	2150.681±73.735^{d}	1432.837±21.556^{g}
96	1431.463±23.091^{g}	3321.738±215.688^{a}	2238.471±94.996^{c}	1434.897±59.169^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different (P < 0.05). (ANOVA followed by HSD Tukey test).

Table 27: Activity of GR (µmol NADPH oxidized/ min/ mg protein) in Muscles of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1153.120±48.386^{h}	1582.499±67.558^{d}	1232.008±39.521^{g}	1155.063±50.745^{h}
48	1157.976±37.466^{h}	1943.314±56.202^{c}	1421.163±57.467^{f}	1154.845±41.637^{h}
72	1151.783±23.901^{h}	2218.761±114.169^{b}	1503.776±65.448^{e}	1159.297±29.503^{h}
96	1156.653±67.436^{h}	2461.887±100.081^{a}	1599.887±80.568^{d}	1154.521±30.841^{h}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different (P < 0.05). (ANOVA followed by HSD Tukey test).

Table 28: Activity of GSH (µmol/ g tissue) in Brain of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1.227 ± 0.068^{h}	2.903 ± 0.142^{d}	1.606 ± 0.247^{g}	1.246 ± 0.041^{h}
48	1.264 ± 0.055^{h}	3.01 ± 0.166^{c}	1.891 ± 0.100^{f}	1.256 ± 0.056^{h}
72	1.241 ± 0.058^{h}	3.456 ± 0.673^{b}	1.937 ± 0.089^{ef}	1.233 ± 0.059^{h}
96	1.282 ± 0.064^{h}	3.822 ± 0.249^{a}	2.152 ± 0.133^{e}	1.254 ± 0.141^{h}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 29: Activity of GSH (µmol/ g tissue) in Gills of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1.076 ± 0.455^{g}	1.333 ± 0.295^{d}	1.157 ± 0.161^{f}	1.072 ± 0.298^{g}
48	1.076 ± 0.129^{g}	1.499 ± 0.231^{c}	1.195 ± 0.098^{ef}	1.078 ± 0.111^{g}
72	1.076 ± 0.121^{g}	1.584 ± 0.203^{b}	1.239 ± 0.137^{e}	1.077 ± 0.112^{g}
96	1.071 ± 0.123^{g}	1.644 ± 0.149^{a}	1.336 ± 0.129^{d}	1.073 ± 0.106^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 30: Activity of GSH (µmol/ g tissue) in Liver of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1.274 ± 0.017^{g}	3.307 ± 0.215^{cd}	2.068 ± 0.043^{f}	1.259 ± 0.016^{g}
48	1.274 ± 0.049^{g}	3.675 ± 0.113^{c}	2.269 ± 0.121^{ef}	1.286 ± 0.031^{g}
72	1.261 ± 0.038^{g}	3.925 ± 0.237^{b}	2.572 ± 0.147^{e}	1.258 ± 0.037^{g}
96	1.306 ± 0.022^{g}	4.173 ± 0.326^{a}	3.214 ± 0.104^{d}	1.279 ± 0.054^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 31: Activity of GSH (µmol/ g tissue) in Muscles of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1.159 ± 0.053^{f}	2.167 ± 0.071^{c}	1.441 ± 0.057^{e}	1.156 ± 0.053^{f}
48	1.157 ± 0.032^{f}	2.578 ± 0.059^{b}	1.494 ± 0.058^{e}	1.154 ± 0.059^{f}
72	1.153 ± 0.048^{f}	2.774 ± 0.141^{ab}	1.578 ± 0.071^{de}	1.153 ± 0.029^{f}
96	1.151 ± 0.045^{f}	2.959 ± 0.122^{a}	1.615 ± 0.077^{d}	1.154 ± 0.029^{f}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 32: Activity of GSH-Px (nmol of GSH oxidized/ min/ mg protein) in Brain of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1.237 ± 0.045^{g}	2.159 ± 0.021^{d}	1.591 ± 0.039^{f}	1.234 ± 0.0103^{g}
48	1.258 ± 0.052^{g}	2.243 ± 0.090^{c}	1.891 ± 0.164^{e}	1.255 ± 0.0282^{g}
72	1.253 ± 0.033^{g}	2.439 ± 0.075^{b}	2.005 ± 0.072^{de}	1.254 ± 0.022^{g}
96	1.297 ± 0.056^{g}	2.603 ± 0.061^{a}	2.247 ± 0.107^{c}	1.284 ± 0.089^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 33: Activity of GSH-Px (nmol of GSH oxidized/ min/ mg protein) in Gills of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1.657 ± 0.107^{i}	2.635 ± 0.151^{d}	1.798 ± 0.061^{h}	1.659 ± 0.062^{i}
48	1.667 ± 0.126^{i}	3.186 ± 0.179^{c}	1.951 ± 0.117^{g}	1.669 ± 0.074^{i}
72	1.679 ± 0.112^{i}	3.560 ± 0.196^{b}	2.171 ± 0.134^{f}	1.671 ± 0.715^{i}
96	1.678 ± 0.099^{i}	3.934 ± 0.301^{a}	2.376 ± 0.096^{e}	1.679 ± 0.063^{i}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 34: Activity of GSH-Px (nmol of GSH oxidized/ min/ mg protein) in Liver of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	2.187 ± 0.055^{e}	4.084 ± 0.231^{bc}	3.493 ± 0.105^{d}	2.144 ± 0.047^{e}
48	2.156 ± 0.065^{e}	4.583 ± 0.143^{b}	3.519 ± 0.069^{d}	2.151 ± 0.031^{e}
72	2.156 ± 0.066^{e}	4.773 ± 0.193^{ab}	3.789 ± 0.065^{cd}	2.153 ± 0.031^{e}
96	2.160 ± 0.062^{e}	4.892 ± 0.298^{a}	3.983 ± 0.117^{c}	2.161 ± 0.079^{e}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 35: Activity of GSH-Px (nmol of GSH oxidized/ min/ mg protein) in Muscles of different groups of fish at different time intervals

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1.656 ± 0.061^{f}	3.175 ± 0.108^{c}	1.985 ± 0.059^{e}	1.651 ± 0.065^{f}
48	1.667 ± 0.052^{f}	3.618 ± 0.104^{b}	1.997 ± 0.053^{e}	1.671 ± 0.035^{f}
72	1.701 ± 0.034^{f}	3.929 ± 0.197^{ab}	2.162 ± 0.069^{de}	1.704 ± 0.009^{f}
96	1.711 ± 0.076^{f}	4.261 ± 0.155^{a}	2.369 ± 0.087^{d}	1.687 ± 0.067^{f}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 36: Activity of Glutathione-S-Transferase (µmol of chloro-2,4-dinitrobenzyne conjugated formed/ min/ mg protein) in Brain

Time	Treatment groups			
(hr)	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1480.320 ± 78.517^{g}	1903.082 ± 73.457^{dc}	1628.579 ± 43.268^{f}	1479.072 ± 34.016^{g}
48	1483.687 ± 109.638^{g}	2227.368 ± 161.101^{c}	1856.692 ± 91.899^{e}	1482.099 ± 55.021^{g}
72	1483.968 ± 72.258^{g}	2655.469 ± 136.538^{b}	2030.650 ± 109.608^{d}	1485.372 ± 21.208^{g}
96	1491.776 ± 102.259^{g}	2964.307 ± 122.388^{a}	2181.288 ± 112.845^{cd}	1486.612 ± 111.808^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 37: Activity of Glutathione-S-Transferase (µmol of chloro-2,4-dinitrobenzyne conjugated formed/ min/ mg protein) in Gills

Time	Treatment groups			
(hr)	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	987.659 ± 65.974^{f}	1083.908 ± 64.562^{e}	1008.974 ± 54.719^{e}	983.805 ± 47.636^{f}
48	984.190 ± 78.128^{f}	1232.029 ± 64.989^{c}	1085.436 ± 68.019^{e}	983.152 ± 48.561^{f}
72	987.166 ± 67.484^{f}	1492.620 ± 98.009^{b}	1178.818 ± 73.111^{d}	984.716 ± 43.168^{f}
96	988.360 ± 62.171^{f}	1752.029 ± 133.623^{a}	1260.237 ± 61.852^{c}	986.011 ± 34.859^{f}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 38: Activity of Glutathione-S-Transferase (µmol of chloro-2,4-dinitrobenzyne conjugated formed/ min/ mg protein) in Liver

Time	Treatment groups			
(hr)	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1756.213 ± 13.165^{i}	3135.194 ± 175.147^{d}	2136.962 ± 95.798^{h}	1758.238 ± 23.401^{i}
48	1753.322 ± 52.619^{i}	4076.190 ± 136.520^{c}	2427.575 ± 73.034^{g}	1756.410 ± 43.075^{i}
72	1761.250 ± 24.811^{i}	4910.765 ± 267.073^{b}	2643.039 ± 85.616^{f}	1757.307 ± 32.307^{i}
96	1763.931 ± 11.794^{i}	5489.824 ± 455.794^{a}	2948.213 ± 123.107^{e}	1752.207 ± 78.068^{i}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 39: Activity of Glutathione-S-Transferase (µmol of chloro-2,4-dinitrobenzyne conjugated formed/ min/ mg protein) in Muscles

Time	Treatment groups			
(hr)	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1377.592 ± 50.221^{g}	2534.021 ± 103.904^{d}	2118.819 ± 68.924^{f}	1376.844 ± 58.635^{g}
48	1379.386 ± 43.719^{g}	2920.042 ± 100.995^{c}	2268.796 ± 97.633^{ef}	1372.667 ± 60.119^{g}
72	1373.279 ± 36.834^{g}	3510.282 ± 189.099^{b}	2358.732 ± 103.460^{e}	1372.827 ± 34.038^{g}
96	1376.002 ± 71.679^{g}	4163.513 ± 181.165^{a}	2581.583 ± 149.859^{d}	1373.981 ± 33.593^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 40: Comparative Specific Activity of Amylase (U/mg) in different groups of fish after

Time	Treatment groups			
(hr)	Control	CYP	Vit C_{100}	Vit C_{200}
24	0.219 ± 0.03^{h}	0.377 ± 0.06^{d}	0.266 ± 0.01^{g}	0.212 ± 0.021^{h}
48	0.218 ± 0.02^{h}	0.405 ± 0.04^{c}	0.301 ± 0.01^{f}	0.214 ± 0.017^{h}
72	0.214 ± 0.02^{h}	0.443 ± 0.05^{b}	0.311 ± 0.01^{ef}	0.216 ± 0.014^{h}
96	0.215 ± 0.02^{h}	0.481 ± 0.04^{a}	0.328 ± 0.01^{e}	0.213 ± 0.047^{h}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 41: Comparative Specific Activity of Cellulase (U/mg) in different groups of fish after

Time	Treatment groups			
(hr)	Control	CYP	Vit C_{100}	Vit C_{200}
24	0.788 ± 0.052^{e}	0.947 ± 0.116^{c}	0.859 ± 0.018^{d}	0.787 ± 0.038^{e}
48	0.784 ± 0.053^{e}	0.964 ± 0.114^{c}	0.866 ± 0.069^{d}	0.788 ± 0.033^{e}
72	0.782 ± 0.054^{e}	1.027 ± 0.136^{b}	0.939 ± 0.021^{cd}	0.783 ± 0.032^{e}
96	0.789 ± 0.052^{e}	1.140 ± 0.114^{a}	0.947 ± 0.031^{c}	0.786 ± 0.023^{e}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 42: Comparative Specific Activity of Protease (U/mg) in different groups of fish

Time	Treatment groups			
(hr)	Control	CYP	Vit C_{100}	Vit C_{200}
24	0.052 ± 0.006^{f}	0.101 ± 0.064^{de}	0.097 ± 0.003^{e}	0.054 ± 0.007^{f}
48	0.052 ± 0.008^{f}	0.115 ± 0.042^{d}	0.107 ± 0.001^{de}	0.053 ± 0.006^{f}
72	0.052 ± 0.006^{f}	0.142 ± 0.017^{b}	0.113 ± 0.002^{d}	0.053 ± 0.005^{f}
96	0.053 ± 0.023^{f}	0.195 ± 0.041^{a}	0.124 ± 0.003^{c}	0.054 ± 0.006^{f}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 43: AST activity (mg protein/ h) in Liver of different groups of fish

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1.556 ± 0.004^{f}	2.149 ± 0.059^{c}	1.779 ± 0.001^{e}	1.556 ± 0.008^{f}
48	1.557 ± 0.001^{f}	2.425 ± 0.078^{b}	1.833 ± 0.167^{de}	$1.556\pm0.103b^{f}$
72	1.557 ± 0.001^{f}	2.636 ± 0.124^{ab}	1.987 ± 0.002^{d}	1.556 ± 0.052^{f}
96	1.556 ± 0.004^{f}	2.961 ± 0.032^{a}	2.092 ± 0.008^{cd}	1.555 ± 0.054^{f}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 44: AST activity (mg protein/ h) in Muscles of different groups of fish

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1.321 ± 0.005^{e}	1.782 ± 0.218^{c}	1.611 ± 0.064^{d}	1.339 ± 0.078^{e}
48	1.339 ± 0.001^{e}	1.954 ± 0.011^{b}	1.654 ± 0.089^{cd}	1.337 ± 0.037^{e}
72	1.343 ± 0.005^{e}	1.994 ± 0.002^{ab}	1.852 ± 0.057^{bc}	1.337 ± 0.007^{e}
96	1.338 ± 0.001^{e}	2.129 ± 0.121^{a}	1.929 ± 0.017^{b}	1.338 ± 0.140^{e}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 45: AST activity (mg protein/ h) in Gills of different groups of fish

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	0.909 ± 0.015^{f}	1.117 ± 0.003^{de}	0.952 ± 0.0204^{f}	0.907 ± 0.009^{f}
48	0.907 ± 0.0203^{f}	1.809 ± 0.085^{c}	1.097 ± 0.027^{e}	0.904 ± 0.027^{f}
72	0.904 ± 0.007^{f}	1.943 ± 0.023^{b}	1.103 ± 0.247^{de}	0.907 ± 0.013^{f}
96	0.907 ± 0.012^{f}	1.987 ± 0.0035^{a}	1.261 ± 0.0404^{d}	0.901 ± 0.016^{f}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 46: ALT activity (mg protein/ h) in Liver of different groups of fish

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	4.61 ± 0.023^{g}	6.317 ± 0.024^{d}	5.487 ± 0.112^{f}	4.61 ± 0.046^{g}
48	4.66 ± 0.0115^{g}	7.882 ± 0.0173^{c}	5.671 ± 0.095^{ef}	4.69 ± 0.046^{g}
72	4.69 ± 0.032^{g}	8.341 ± 0.314^{b}	5.817 ± 0.011^{e}	4.68 ± 0.017^{g}
96	4.65 ± 0.048^{g}	8.847 ± 0.824^{a}	6.297 ± 0.235^{de}	4.63 ± 0.082^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 47: ALT activity (mg protein/ h) in Muscles of different groups of fish

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	3.36 ± 0.005^{f}	4.28 ± 0.0115^{cd}	3.573 ± 0.0671^{e}	3.31 ± 0.01^{f}
48	3.32 ± 0.006^{f}	4.87 ± 0.0231^{c}	3.973 ± 0.023^{d}	3.32 ± 0.029^{f}
72	3.39 ± 0.011^{f}	5.58 ± 0.012^{b}	4.44 ± 0.023^{c}	3.40 ± 0.029^{f}
96	3.34 ± 0.006^{f}	6.69 ± 0.005^{a}	5.52 ± 0.0289^{b}	3.34 ± 0.0115^{f}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 48: ALT activity (mg protein/ h) in Gills of different groups of fish

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	1.78 ± 0.067^{f}	2.43 ± 0.035^{cd}	2.077 ± 0.088^{e}	1.77 ± 0.029^{f}
48	1.75 ± 0.145^{f}	2.91 ± 0.078^{bc}	2.133 ± 0.034^{de}	1.71 ± 0.009^{f}
72	1.79 ± 0.064^{f}	3.63 ± 0.111^{b}	2.287 ± 0.177^{d}	1.77 ± 0.018^{f}
96	1.76 ± 0.031^{f}	3.97 ± 0.023^{a}	2.697 ± 0.157^{c}	1.78 ± 0.091^{f}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 49: GDH activity (mg protein/ h) in Liver of different groups of fish

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	0.76 ± 0.012^{e}	0.837 ± 0.068^{cd}	0.81 ± 0.046^{d}	0.76 ± 0.029^{e}
48	0.77 ± 0.011^{e}	0.921 ± 0.029^{bc}	0.83 ± 0.046^{cd}	0.76 ± 0.006^{e}
72	0.77 ± 0.035^{e}	0.973 ± 0.011^{b}	0.89 ± 0.041^{c}	0.76 ± 0.046^{e}
96	0.77 ± 0.058^{e}	1.033 ± 0.031^{a}	0.89 ± 0.052^{c}	0.76 ± 0.006^{e}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 50: GDH activity (mg protein/ h) in Muscles of different groups of fish

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	0.43 ± 0.011^{f}	0.49 ± 0.023^{de}	0.45 ± 0.029^{ef}	0.43 ± 0.017^{f}
48	0.44 ± 0.029^{f}	0.50 ± 0.017^{d}	0.47 ± 0.035^{e}	0.43 ± 0.011^{f}
72	0.44 ± 0.006^{f}	0.64 ± 0.011^{b}	0.58 ± 0.023^{c}	0.43 ± 0.017^{f}
96	0.44 ± 0.017^{f}	0.69 ± 0.011^{a}	0.61 ± 0.075^{bc}	0.44 ± 0.069^{f}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 51: GDH activity (mg protein/ h) in Gills of different groups of fish

Time (hr)	Treatment groups			
	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	0.37 ± 0.011^{f}	0.43 ± 0.035^{de}	0.41 ± 0.017^{e}	0.36 ± 0.017^{f}
48	0.37 ± 0.012^{f}	0.49 ± 0.041^{c}	0.43 ± 0.017^{de}	0.37 ± 0.0104^{f}
72	0.37 ± 0.011^{f}	0.56 ± 0.0173^{b}	0.45 ± 0.023^{d}	0.37 ± 0.0321^{f}
96	0.37 ± 0.017^{f}	0.63 ± 0.0289^{a}	0.49 ± 0.035^{c}	0.37 ± 0.0231^{f}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 52: LDH activity (mg protein/ h) in Liver of different groups of fish

Time	Treatment groups			
(hr)	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	0.221 ± 0.013^{g}	0.449 ± 0.002^{e}	0.398 ± 0.002^{f}	0.223 ± 0.004^{g}
48	0.219 ± 0.001^{g}	0.622 ± 0.012^{c}	0.433 ± 0.002^{ef}	0.218 ± 0.015^{g}
72	0.220 ± 0.005^{g}	0.821 ± 0.009^{b}	0.489 ± 0.001^{de}	0.221 ± 0.009^{g}
96	0.223 ± 0.006^{g}	0.946 ± 0.011^{a}	0.508 ± 0.004^{d}	0.221 ± 0.007^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 53: LDH activity (mg protein/ h) in Muscles of different groups of fish

Time	Treatment groups			
(hr)	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	0.191 ± 0.001^{e}	0.207 ± 0.002^{d}	0.199 ± 0.001^{de}	0.191 ± 0.001^{e}
48	0.191 ± 0.001^{e}	0.221 ± 0.002^{c}	0.211 ± 0.003^{d}	0.191 ± 0.005^{e}
72	0.190 ± 0.002^{e}	0.239 ± 0.001^{b}	0.223 ± 0.004^{c}	0.191 ± 0.001^{e}
96	0.191 ± 0.001^{e}	0.268 ± 0.003^{a}	0.241 ± 0.001^{b}	0.191 ± 0.004^{e}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 54: LDH activity (mg protein/ h) in Gills of different groups of fish

Time	Treatment groups			
(hr)	Control	CYP	CYP + Vit C_{100}	CYP + Vit C_{200}
24	0.119 ± 0.003^{g}	0.137 ± 0.004^{e}	0.124 ± 0.003^{f}	0.120 ± 0.005^{g}
48	0.121 ± 0.005^{g}	0.149 ± 0.004^{d}	0.137 ± 0.001^{e}	0.119 ± 0.002^{g}
72	0.120 ± 0.004^{g}	0.171 ± 0.005^{b}	0.151 ± 0.002^{d}	0.120 ± 0.009^{g}
96	0.120 ± 0.007^{g}	0.201 ± 0.006^{a}	0.159 ± 0.002^{c}	0.120 ± 0.002^{g}

Data are represented as Mean±SE (n = 3). Means followed by different letters are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 55: Genotoxic damage in peripheral erythrocyte of different groups of rohu after 24 hr

Parameters	Treatment groups			
	Control	CYP	CYP+Vit C_{100}	CYP+Vit C_{200}
Comets/120cells	20.01 ± 0.13^{c}	27.78 ± 0.25^{a}	25.78 ± 0.32^{b}	20.11 ± 0.41^{c}
Comet Length	42.0 ± 4.41^{b}	47.13 ± 3.38^{a}	46.57 ± 3.63^{a}	41.60 ± 5.04^{b}
Tail Length	7.73 ± 1.16^{b}	15.63 ± 1.81^{a}	9.33 ± 1.05^{b}	8.20 ± 1.44^{b}
Head Length	34.10 ± 3.72^{b}	32.40 ± 2.55^{c}	37.60 ± 3.17^{a}	34.80 ± 4.43^{b}
% DNA in Head	91.22 ± 0.55^{a}	80.45 ± 3.09^{c}	85.82 ± 0.93^{b}	$92.89\pm0.78a^{a}$
% DNA in Tail	8.78 ± 0.57^{c}	19.19 ± 3.12^{a}	14.11 ± 0.97^{b}	7.11 ± 0.81^{c}
Tail Movement	0.85 ± 0.11^{c}	1.88 ± 0.59^{a}	1.02 ± 0.14^{b}	0.79 ± 0.10^{c}
OTM*	0.89 ± 0.17^{c}	2.52 ± 0.24^{a}	1.13 ± 0.16^{b}	0.88 ± 0.17^{c}

Data are represented as Mean±SE (n = 3). Means followed by different letters within the row are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 56: Genotoxic damage in peripheral erythrocyte of different groups of rohu after 48 hr

Parameters	Treatment groups			
	Control	CYP	CYP+Vit C_{100}	CYP+Vit C_{200}
Comets/120cells	19.91 ± 0.22^{c}	33.18 ± 0.43^{a}	28.78 ± 0.92^{b}	20.45 ± 0.32^{c}
Comet Length	44.70 ± 4.96^{b}	48.97 ± 3.79^{a}	48.27 ± 4.55^{a}	43.67 ± 3.71^{b}
Tail Length	5.73 ± 0.48^{c}	23.87 ± 2.79^{a}	10.40 ± 1.58^{b}	5.10 ± 1.04^{c}
Head Length	38.97 ± 4.48^{b}	25.10 ± 2.79^{c}	39.27 ± 3.97^{a}	38.57 ± 2.67^{b}
% DNA in Head	89.89 ± 0.55^{a}	70.58 ± 4.13^{c}	83.46 ± 1.49^{b}	90.98 ± 0.64^{a}
% DNA in Tail	10.11 ± 0.52^{c}	29.46 ± 4.12^{a}	16.09 ± 1.55^{b}	9.12 ± 0.69^{c}
Tail Movement	0.93 ± 0.13^{c}	1.98 ± 0.16^{a}	1.26 ± 0.15^{b}	0.86 ± 0.11^{c}
OTM*	0.89 ± 0.17^{c}	2.92 ± 0.26^{a}	1.25 ± 0.19^{b}	0.91 ± 0.17^{c}

Data are represented as Mean±SE (n = 3). Means followed by different letters within the row are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 57: Genotoxic damage in peripheral erythrocyte of different groups of rohu after 72 hr

Parameters	Treatment groups			
	Control	CYP	CYP+Vit C_{100}	CYP+Vit C_{200}
Comets/120cells	20.97±0.51^{c}	38.47±0.74^{a}	32.61±0.57^{b}	21.13±0.29^{c}
Comet Length	42.60±5.04^{c}	52.53±3.80^{a}	49.50±2.54^{b}	43.83±3.73^{c}
Tail Length	7.30±1.33^{c}	25.47±2.58^{a}	13.36±1.52^{b}	8.83±0.85^{c}
Head Length	36.27±4.63^{a}	27.07±3.11^{c}	34.93±2.97^{b}	36.63±2.50^{a}
% DNA in Head	91.32±0.56^{a}	65.75±3.92^{c}	82.92±1.19^{b}	92.89±0.63^{a}
% DNA in Tail	8.61±.57^{c}	34.32±3.91^{a}	17.08±1.34^{b}	7.10±0.63^{c}
Tail Movement	0.89±0.10^{c}	2.01±0.10^{a}	1.46±0.19^{b}	0.93±0.11^{c}
OTM*	0.88±0.17^{c}	3.04±0.25^{a}	1.41±0.21^{b}	0.91±0.17^{c}

Data are represented as Mean±SE (n = 3). Means followed by different letters within the row are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

Table 58: Genotoxic damage in peripheral erythrocyte of different groups of rohu after 96 hr

Parameters	Treatment groups			
	Control	CYP	CYP+Vit C_{100}	CYP+Vit C_{200}
Comets/120cells	20.78±0.43^{c}	41.47±0.87^{a}	36.61±1.01^{b}	21.43±0.38^{c}
Comet Length	44.83±3.72^{c}	57.30±4.00^{a}	51.30±4.09^{b}	44.67±3.69^{c}
Tail Length	9.66±0.79^{c}	29.07±2.87^{a}	16.87±2.67^{b}	9.33±1.11^{c}
Head Length	35.17±3.05^{b}	28.23±3.42^{c}	37.60±4.22^{a}	35.34±3.17^{b}
% DNA in Head	90.85±0.56^{a}	63.25±4.03^{c}	79.72±1.42^{b}	91.82±0.93^{a}
% DNA in Tail	9.15±0.57^{c}	36.81±4.02^{a}	20.42±1.55^{b}	8.12±0.97^{c}
Tail Movement	0.86±0.11^{c}	2.19±0.21^{a}	1.42±0.14^{b}	0.79±0.10^{c}
OTM*	1.09±0.17^{c}	3.12±0.23^{a}	1.88±0.17^{b}	1.13±0.16^{c}

Data are represented as Mean±SE (n = 3). Means followed by different letters within the row are significantly different ($P < 0.05$). (ANOVA followed by HSD Tukey test).

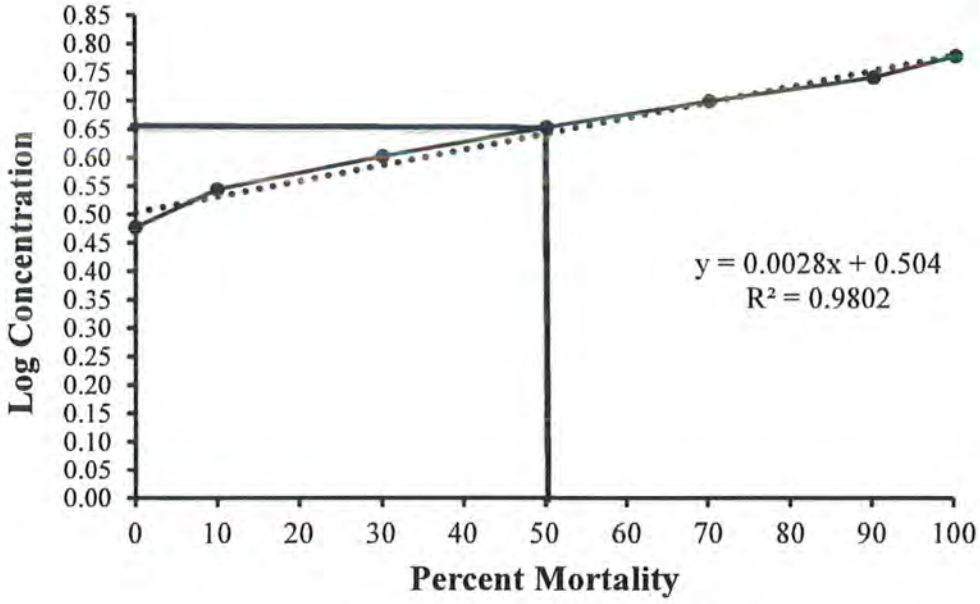

Fig 1: Toxicity evaluation of CYP against rohu, *Labeo rohita*

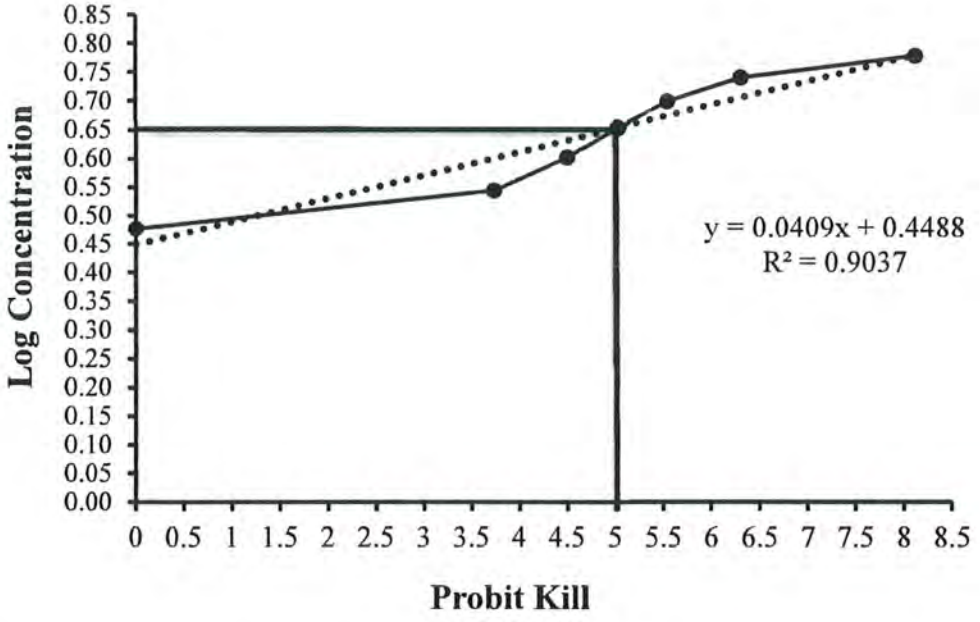

Fig 2: Toxicity evaluation of CYP against rohu, *Labeo rohita*

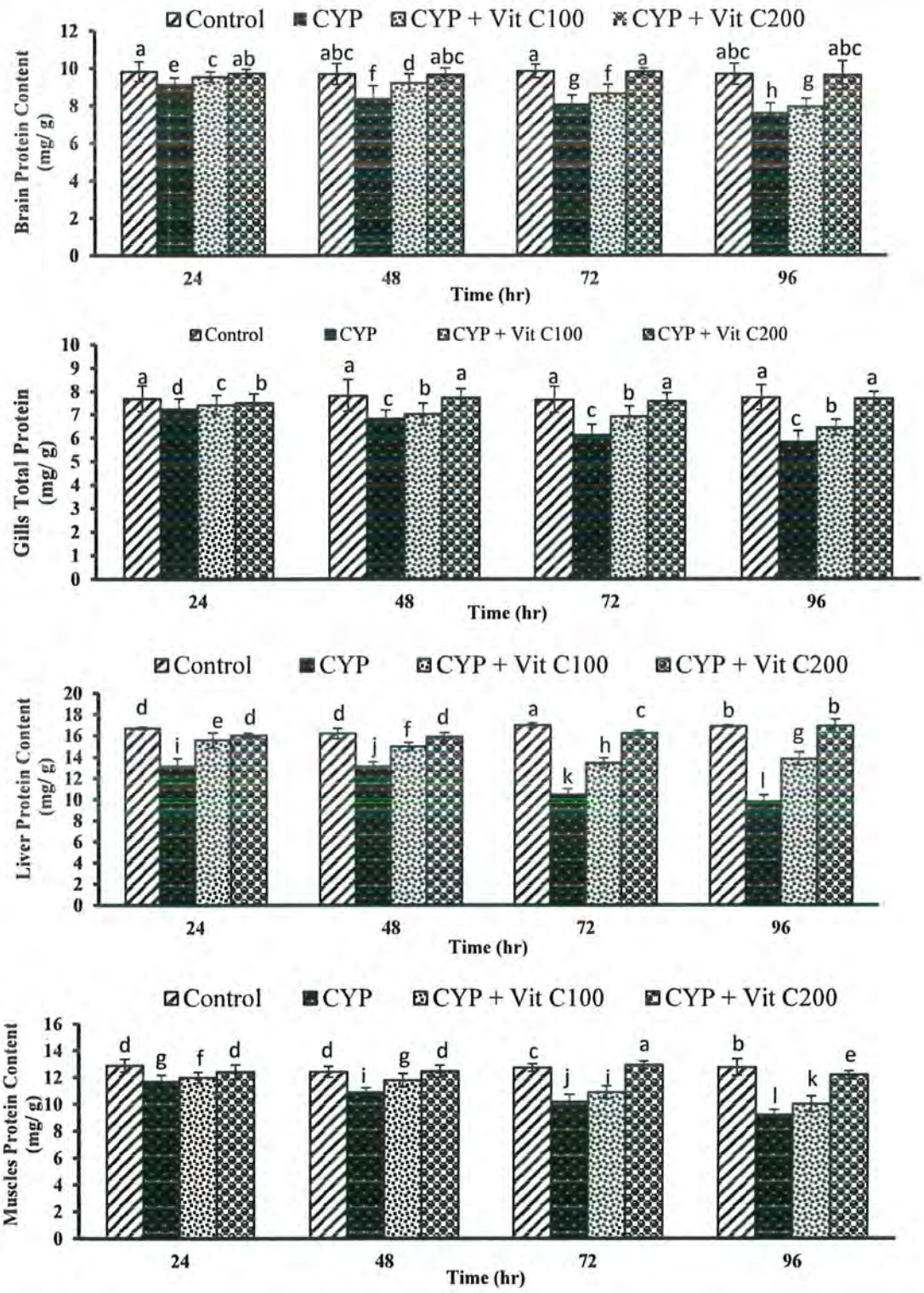

Fig 3: Total proteins content (mg/ g) in brain, gills, liver and muscles tissues of rohu, *Labeo rohita*, after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n=3). Means with different letters are significantly different (P<0.05).

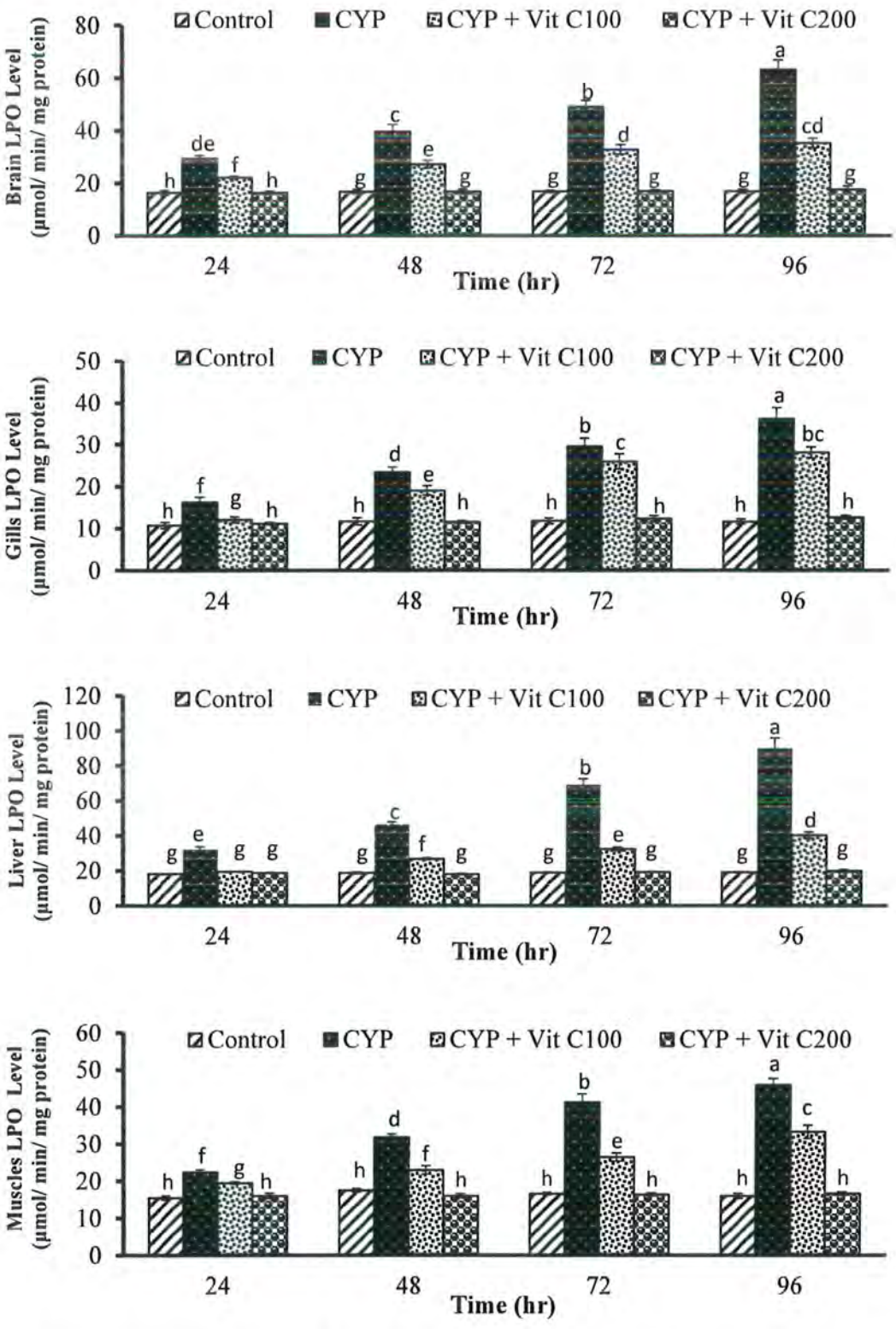

Fig 4: Lipid peroxidation (LPO) Level (µmol/ min/ mg protein) in brain, gills, liver and muscles tissues of rohu, *Labeo rohita*, after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n=3). Means with different letters are significantly different ($P<0.05$).

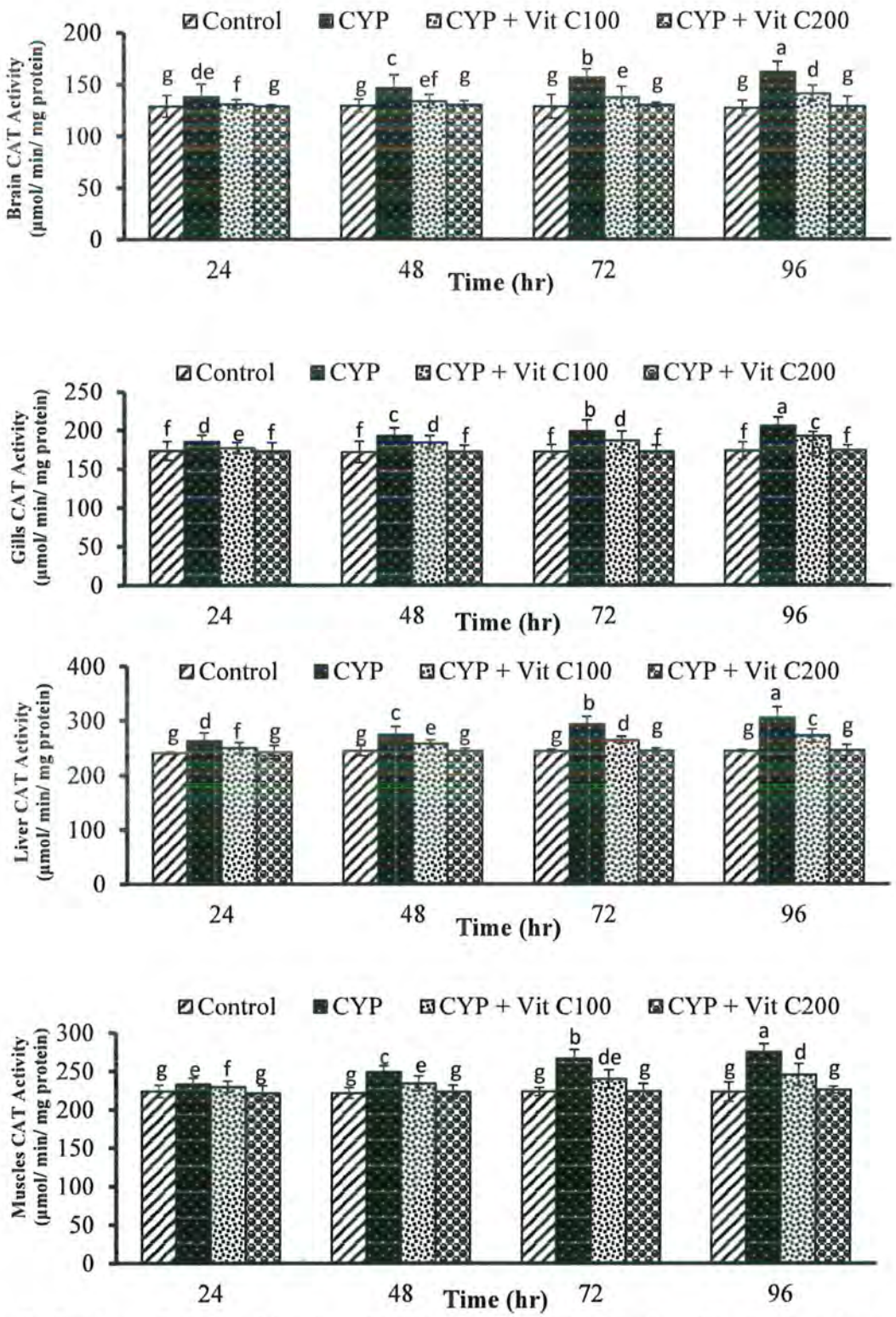

Fig 5: Catalase (CAT) activity (µmol/ min/ mg protein) in brain, gills, liver and muscles tissues of rohu, *Labeo rohita*, after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

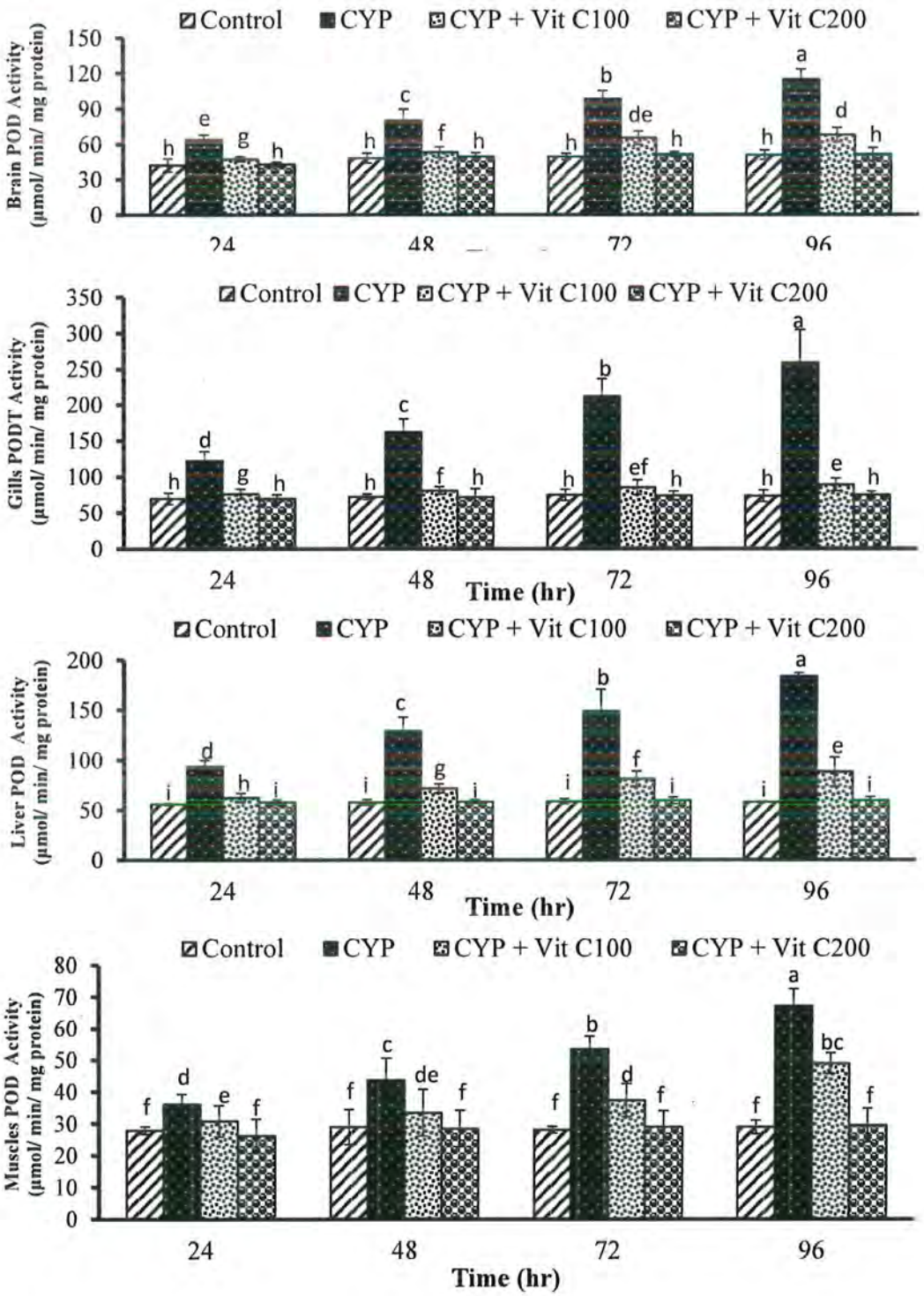

Fig 6: Peroxidase (POD) activity (µmol/ min/ mg protein) in brain, gills, liver and muscle of rohu, *Labeo rohita*, after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

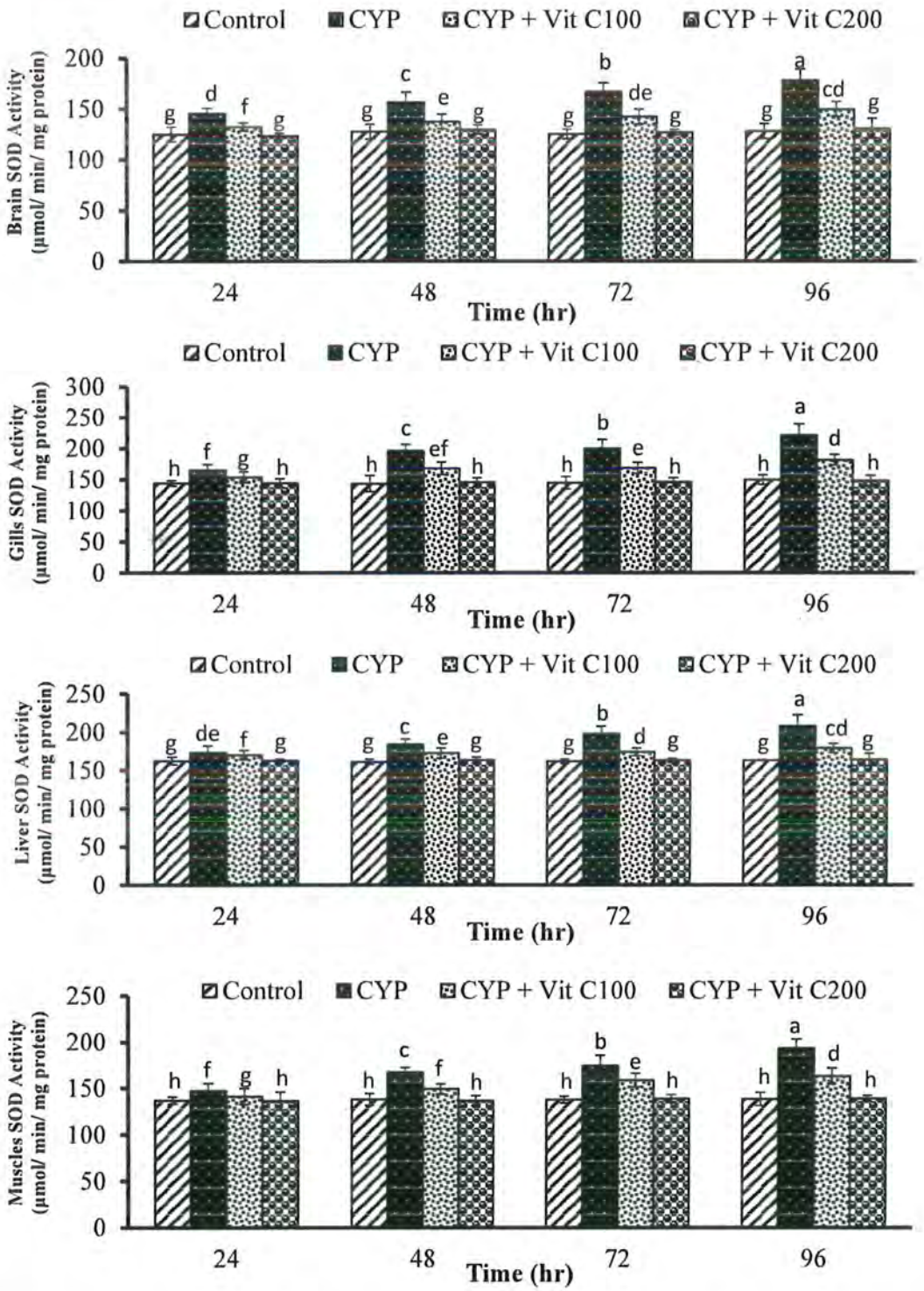

Fig 7: Superoxide Dismutase (SOD) activity (µmol/ min/ mg protein) in brain, gills, liver and muscle of rohu, *Labeo rohita*, after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

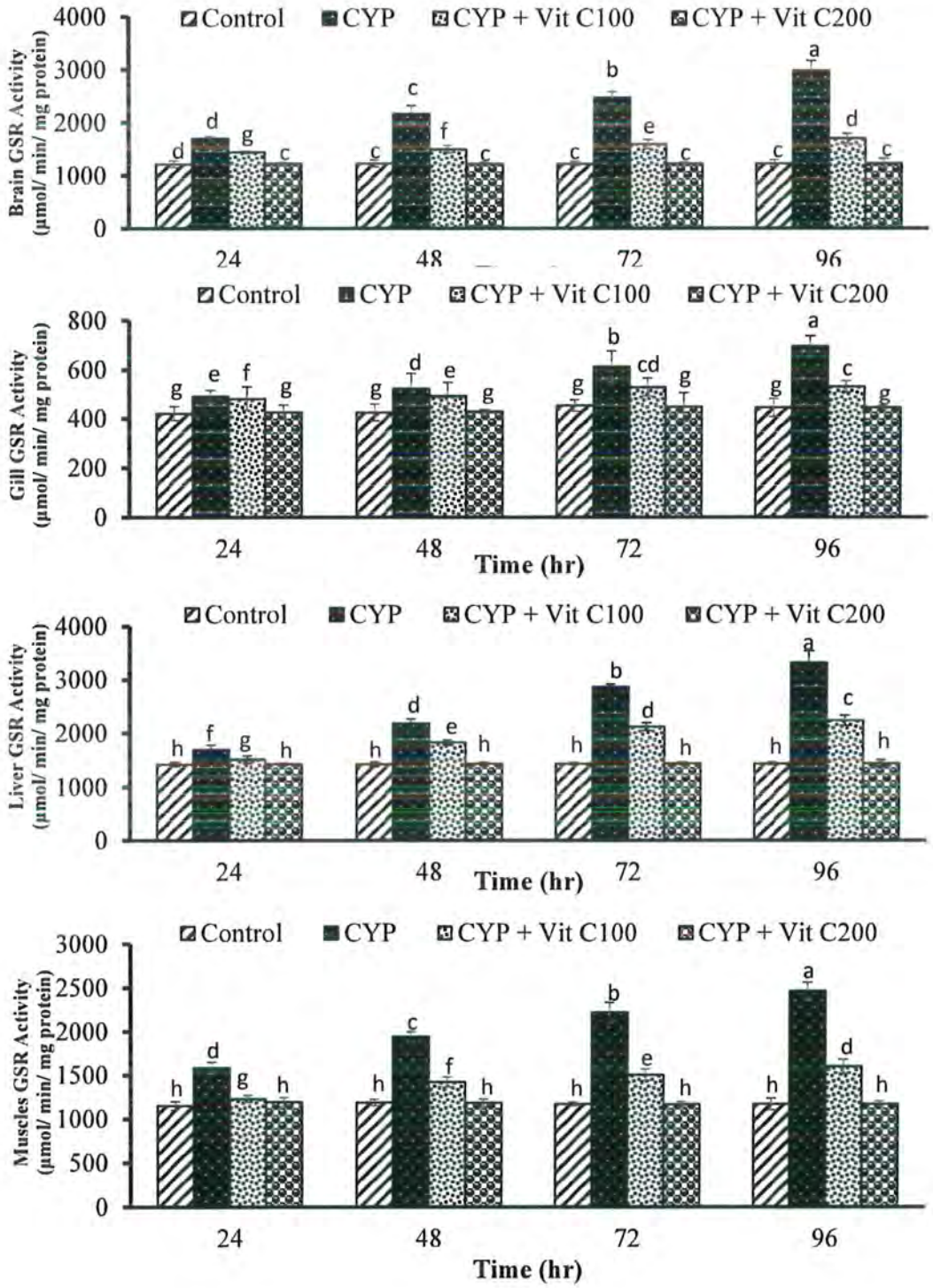

Fig 8: Glutathione Reductase (GR) activity (µmol/ min/ mg protein) in brain, gills, liver and muscles of rohu, *Labeo rohita*, after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

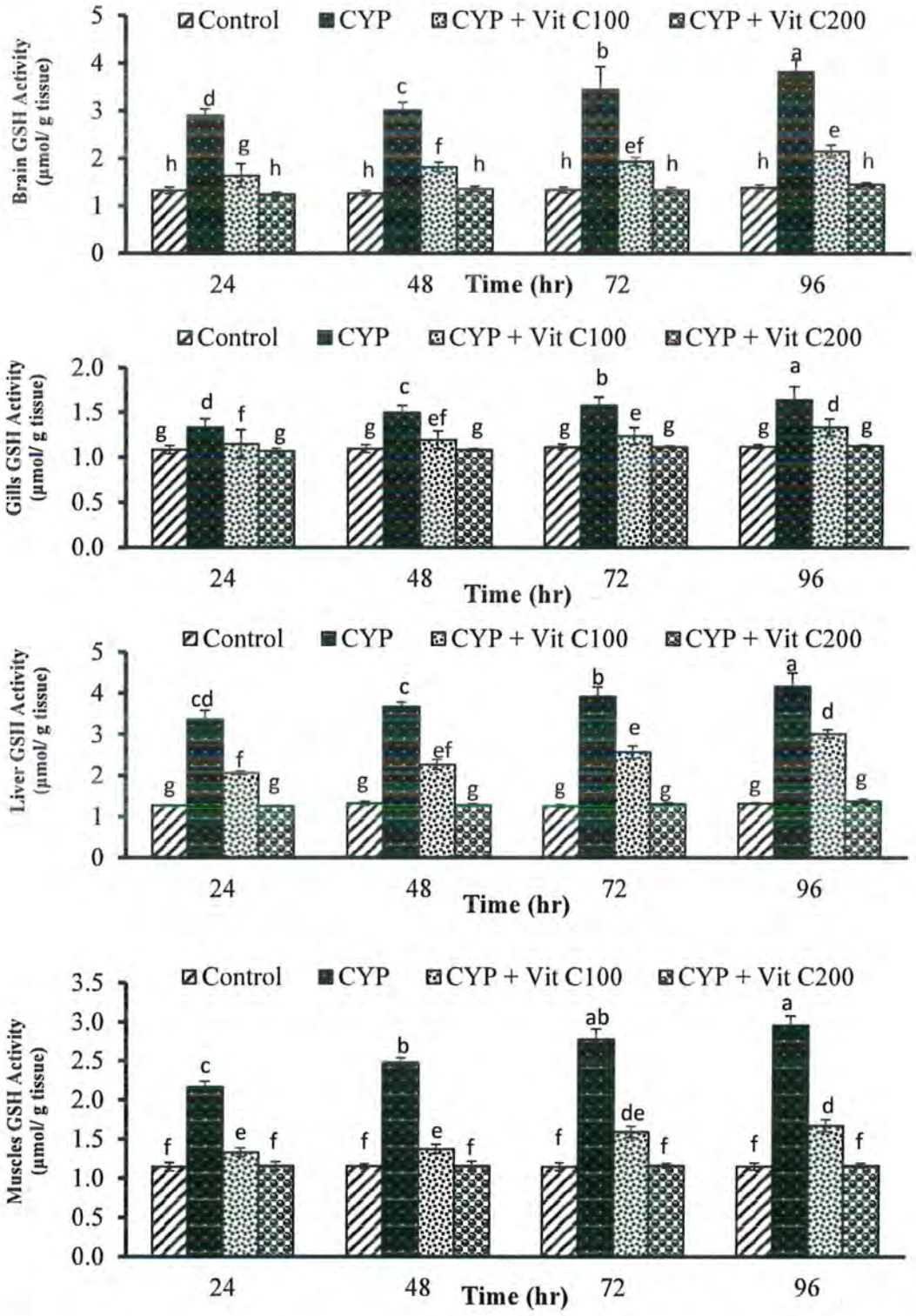

Fig 9: Reduced Glutathione Contents (GSH) activity (µmol/ g tissue) in brain, gills, liver and muscles tissues of rohu, *Labeo rohita*, after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

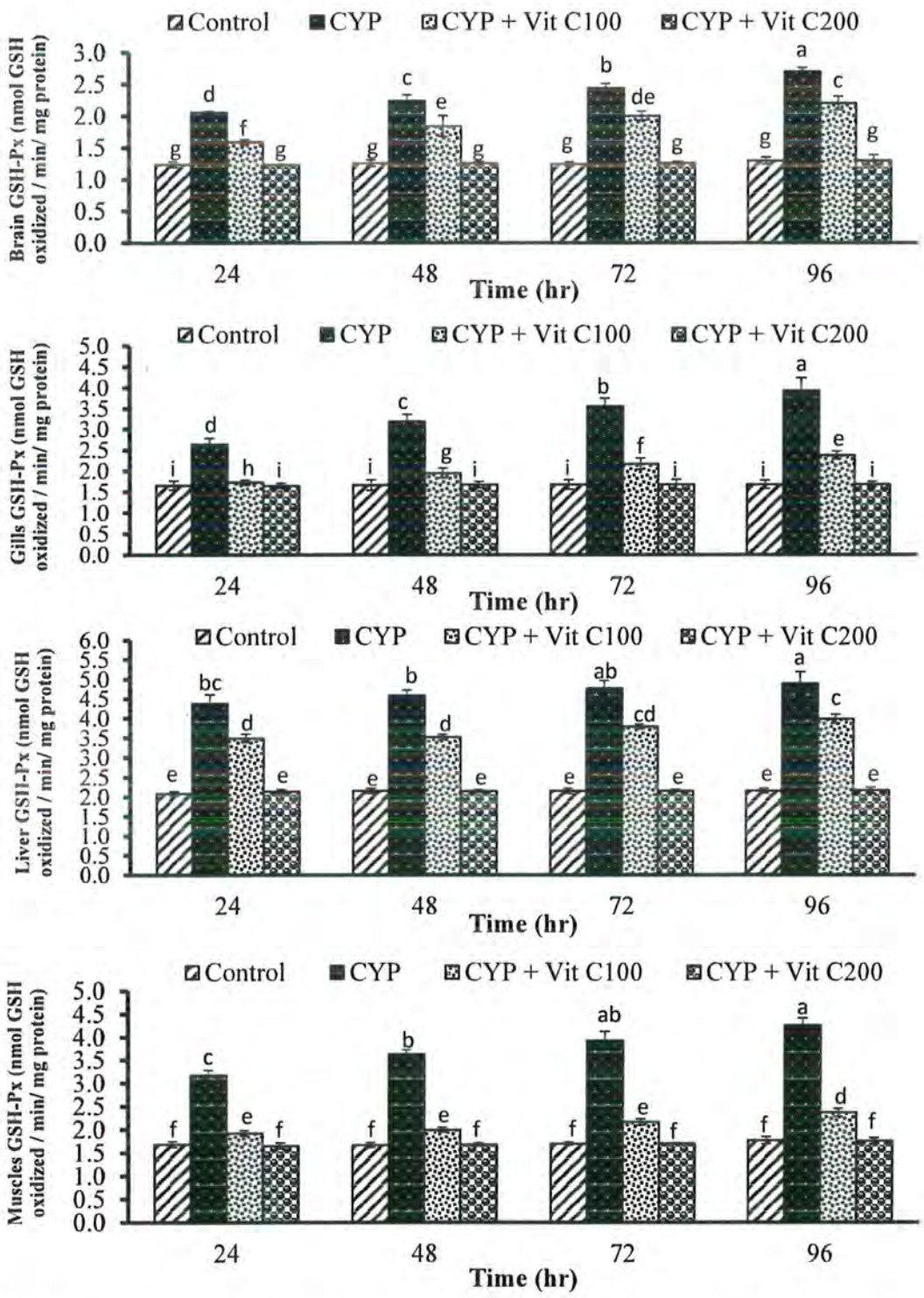

Fig 10: Glutathione Peroxidase (GSH-Px) activity (nmol GSH oxidized/ min/ mg protein) in liver, muscles and gills tissues of rohu, *Labeo rohita*, after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

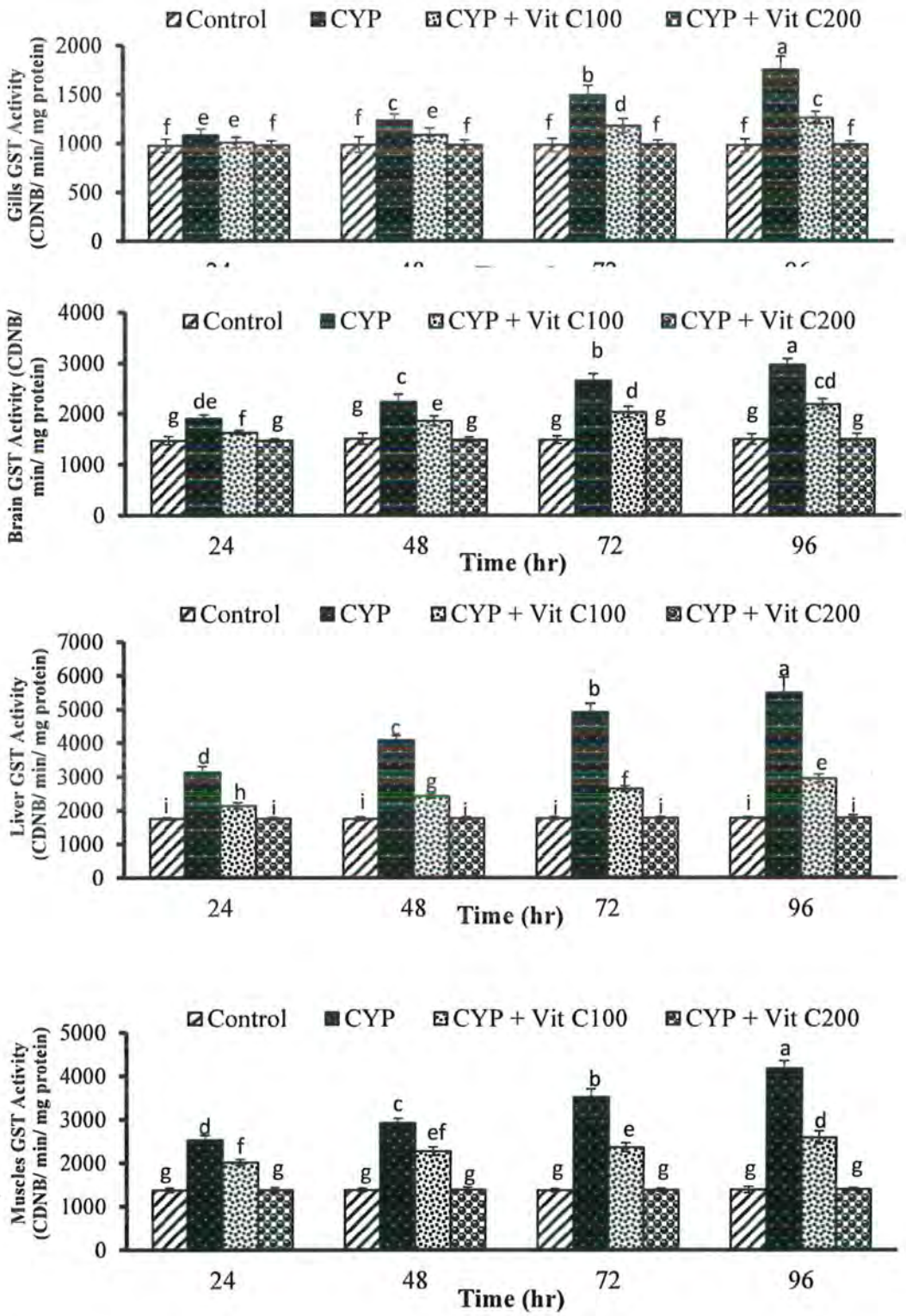

Fig 11: Glutathione-S-transferase (GST) activity (µmol CDNB conjugate formed/ min/ mg protein) in brain, gills, liver and muscles tissues of rohu, *Labeo rohita*, after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

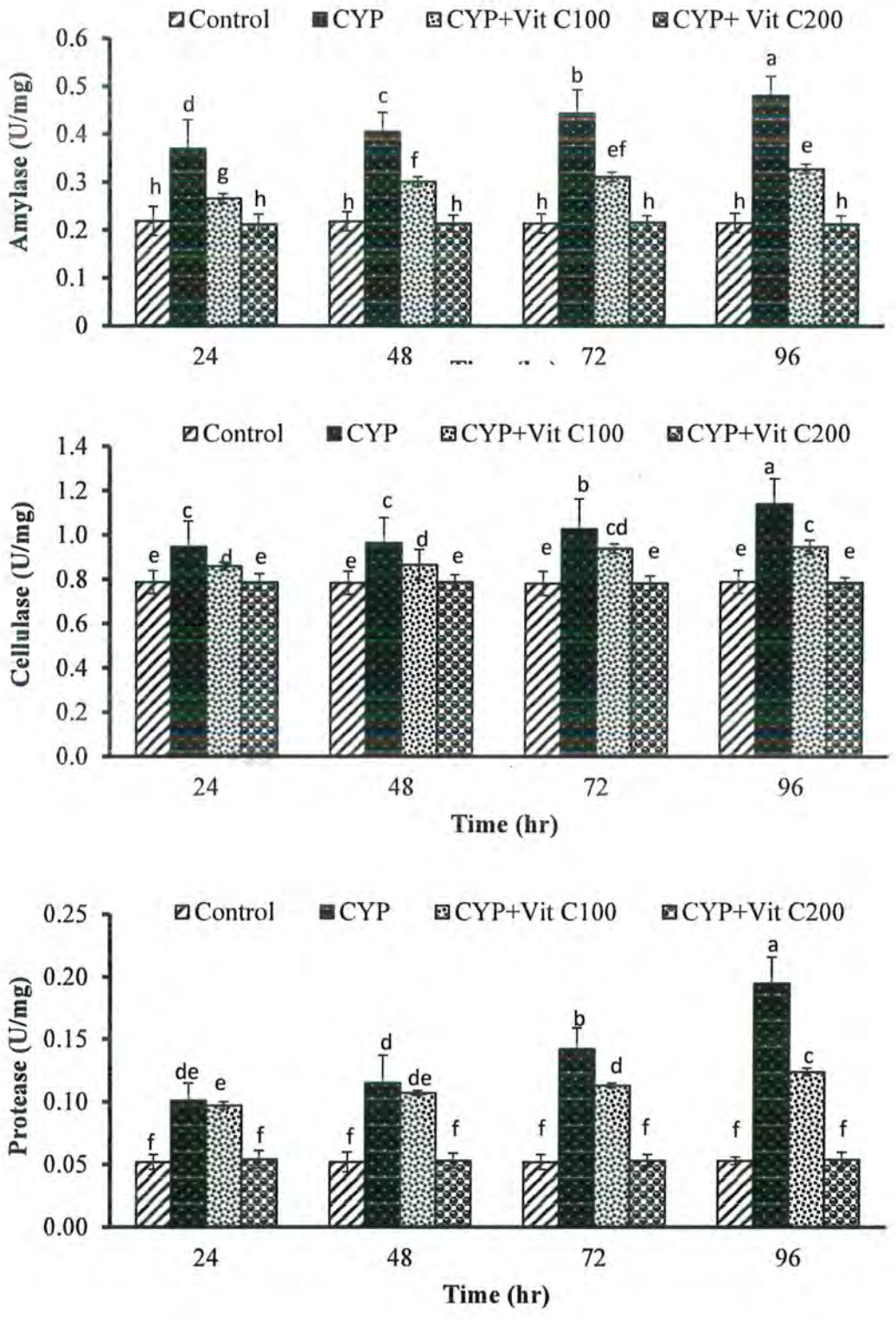

Fig 12: Activity (U/mg) of Digestive enzymes (Amylase, Cellulase and Protease) after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

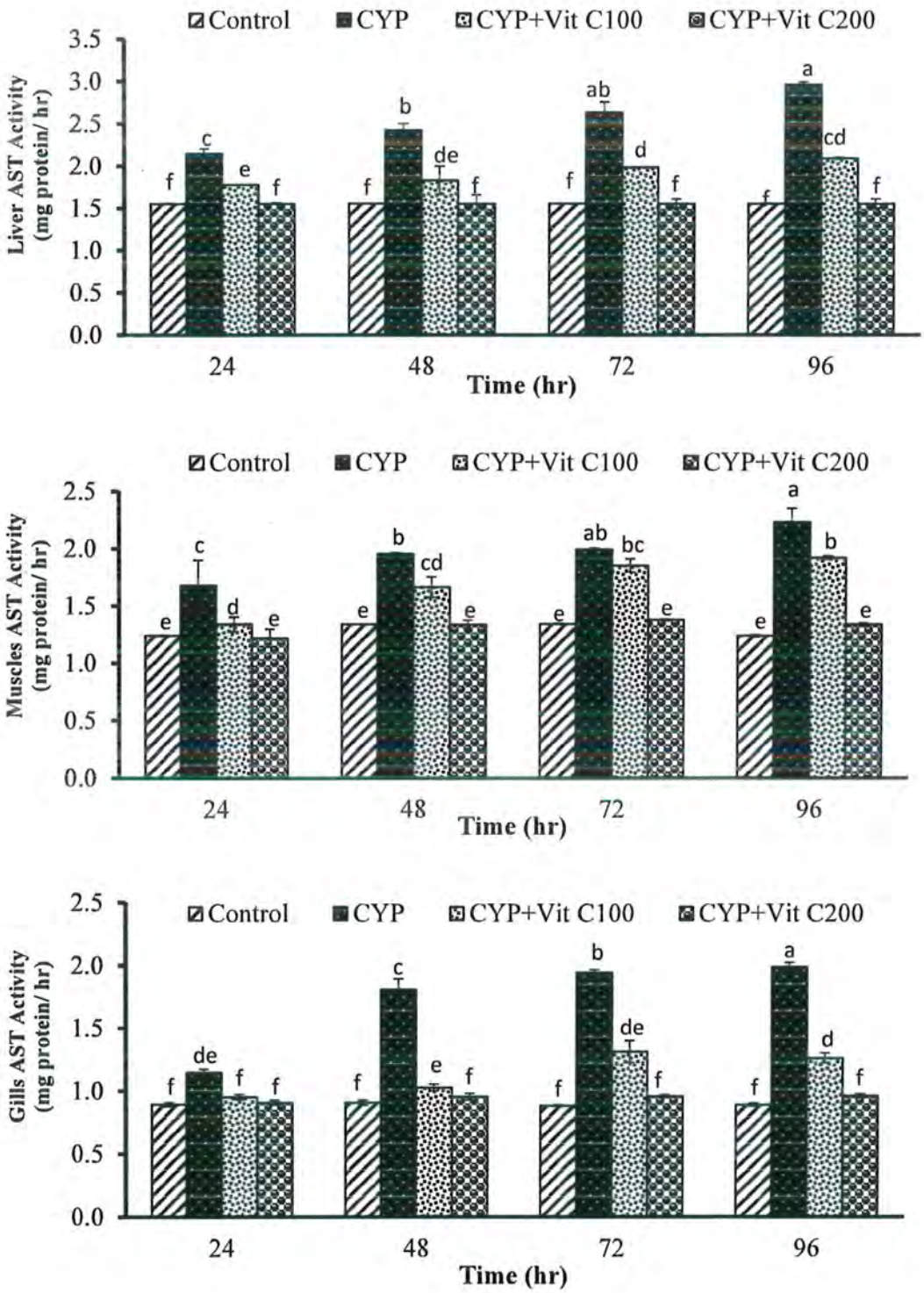

Fig 13: Activity (mg protein/ hr) of Aspartate Aminotransferase (AST) in liver, muscles and gills tissues of rohu, *Labeo rohita*, after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

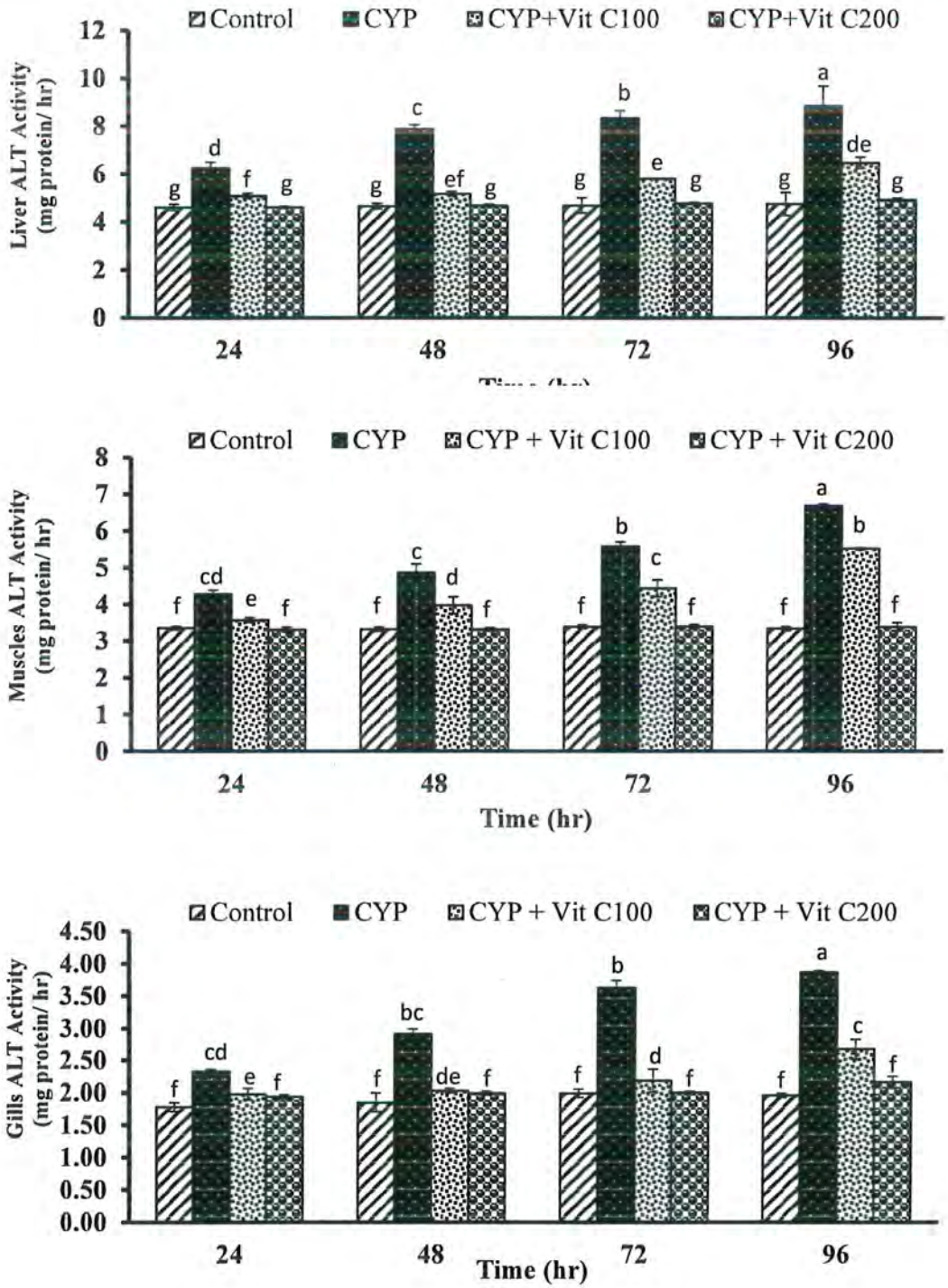

Fig 14: Activity (mg protein/ hr) of Alanine Aminotransferase (ALT) in liver, muscles and gills tissues of rohu, *Labeo rohita*, after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n=3). Means with different letters are significantly different ($P<0.05$).

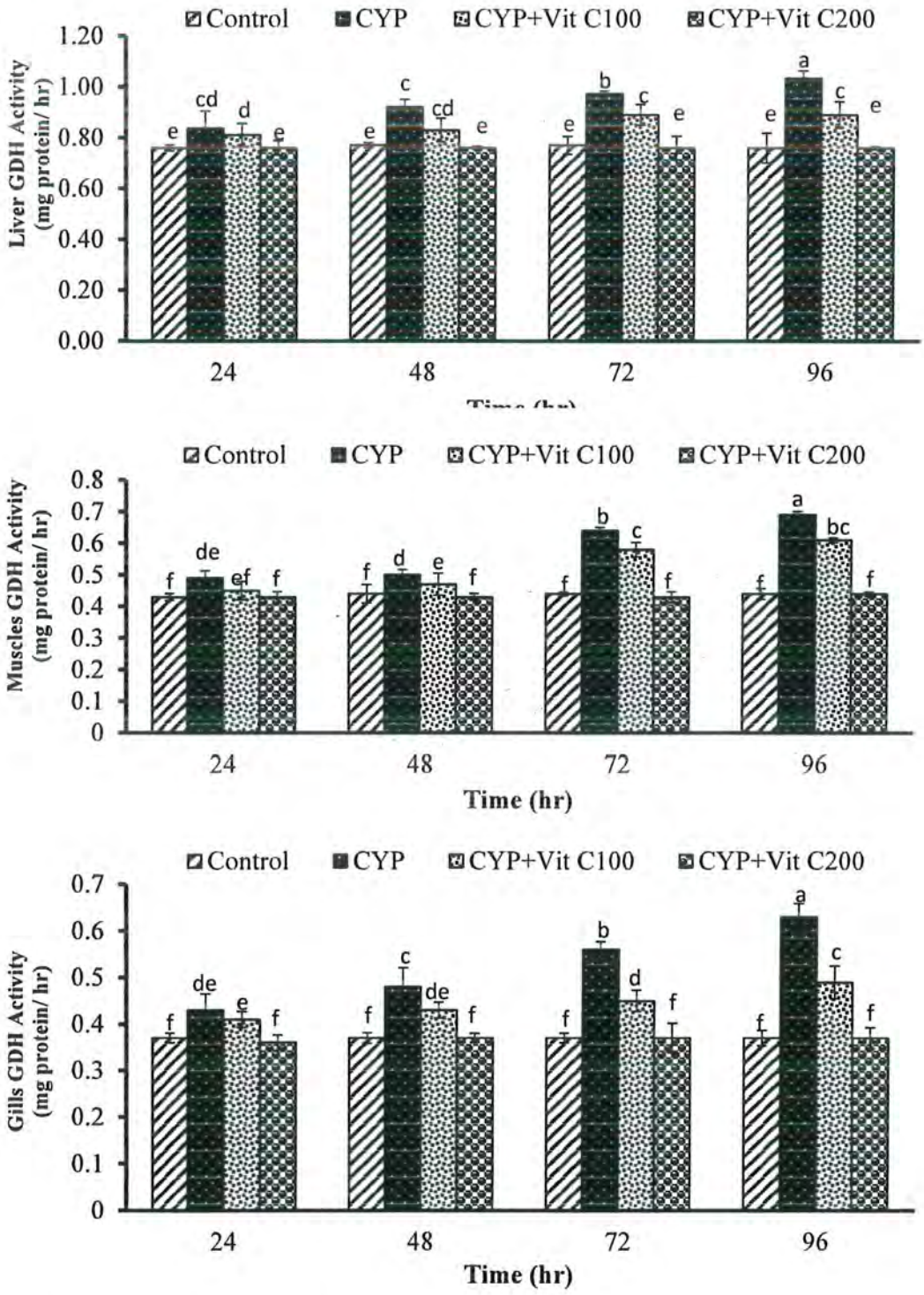

Fig 15: Activity (mg protein/ hr) of Glutamate Dehydrogenase (GDH) in liver, muscles and gills tissues of rohu, *Labeo rohita*, after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

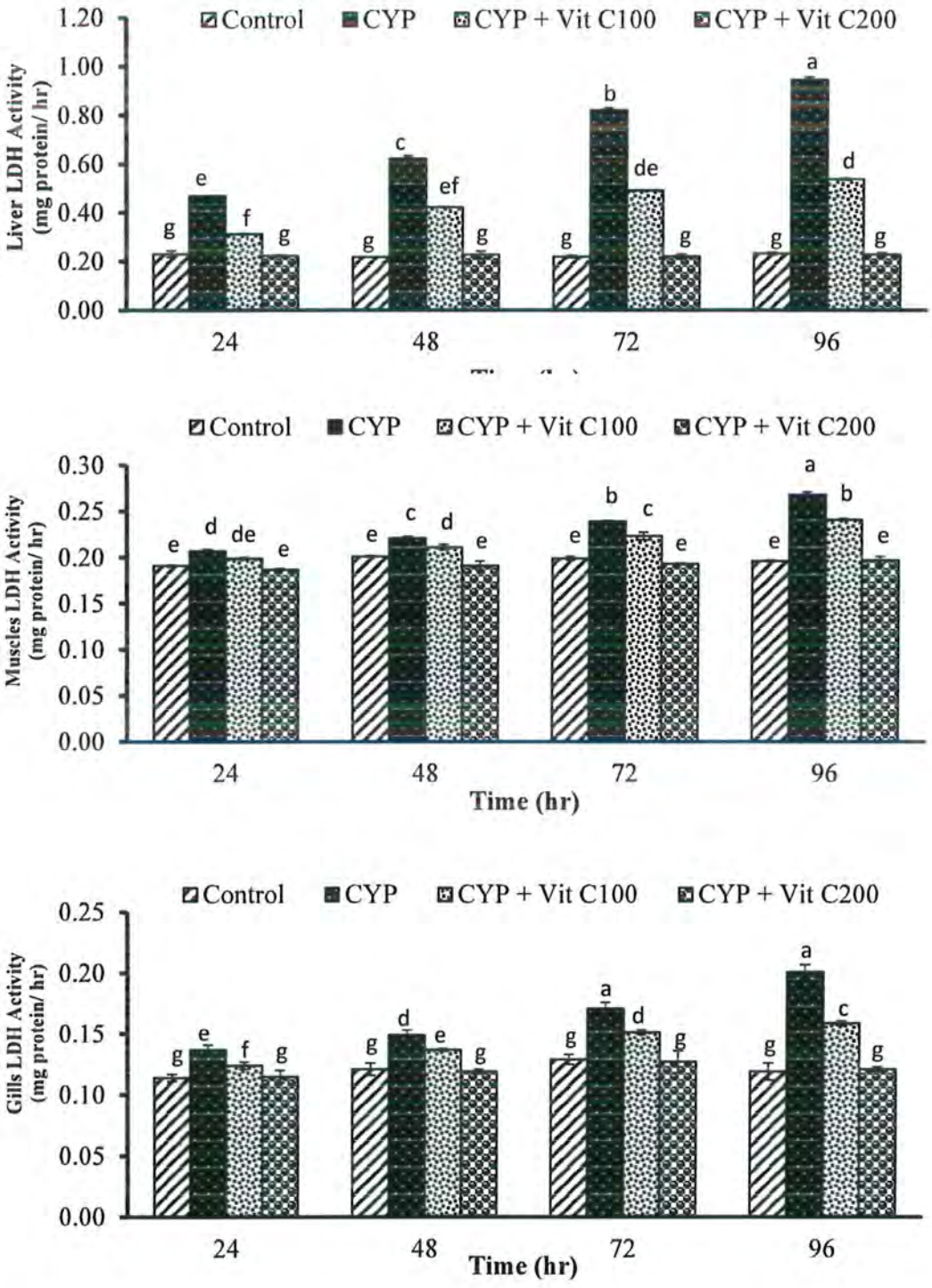

Fig 16: Activity (mg protein/ hr) of Lactate Dehydrogenase (LDH) in liver, muscles and gills tissues of rohu, *Labeo rohita*, after exposure to acute concentration of CYP and prior feeding of Vit C. Data are mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

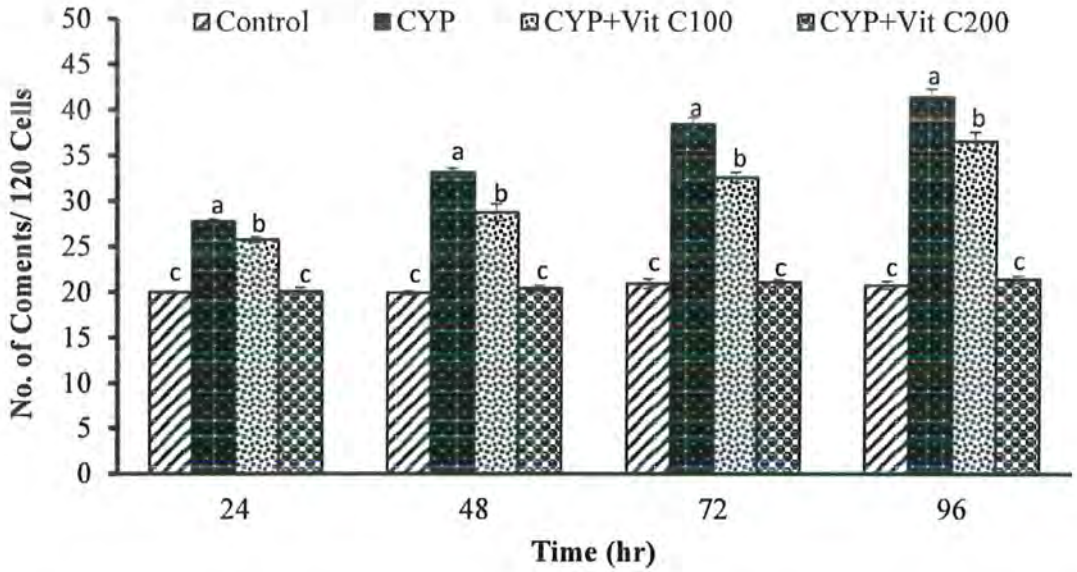

Fig 17. Number of comets per 120 cells observed in peripheral erythrocytes of rohu at different time intervals after exposure of LC_{50} of CYP and ameliorative role of Vit C Data are presented as mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

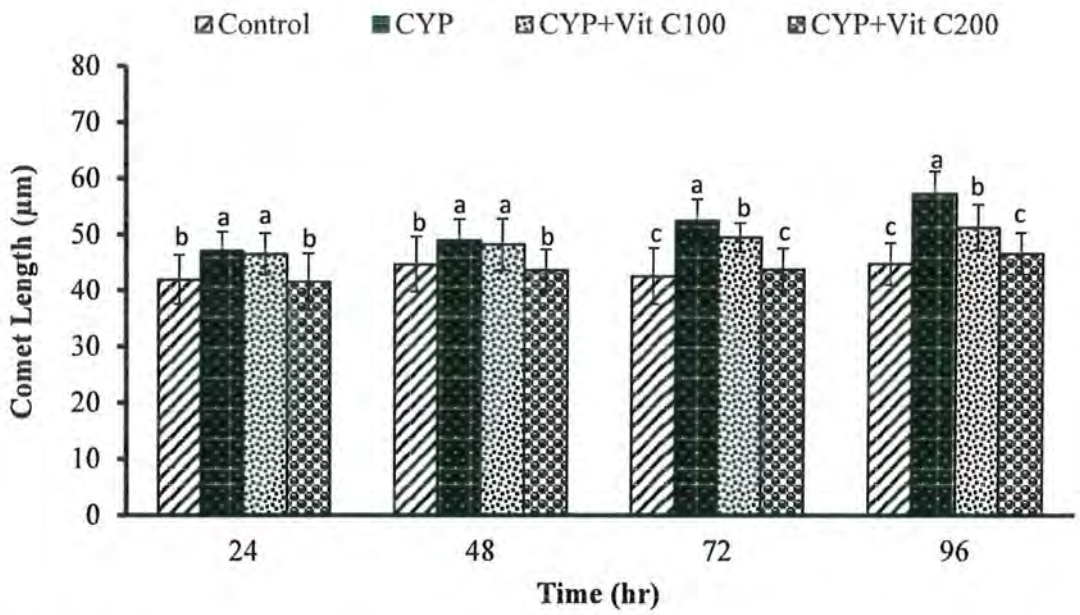

Fig 18: Length of comets (µm) formed in peripheral erythrocytes of rohu at different time intervals after exposure of LC_{50} of CYP and ameliorative role of Vit C. Data are presented as mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

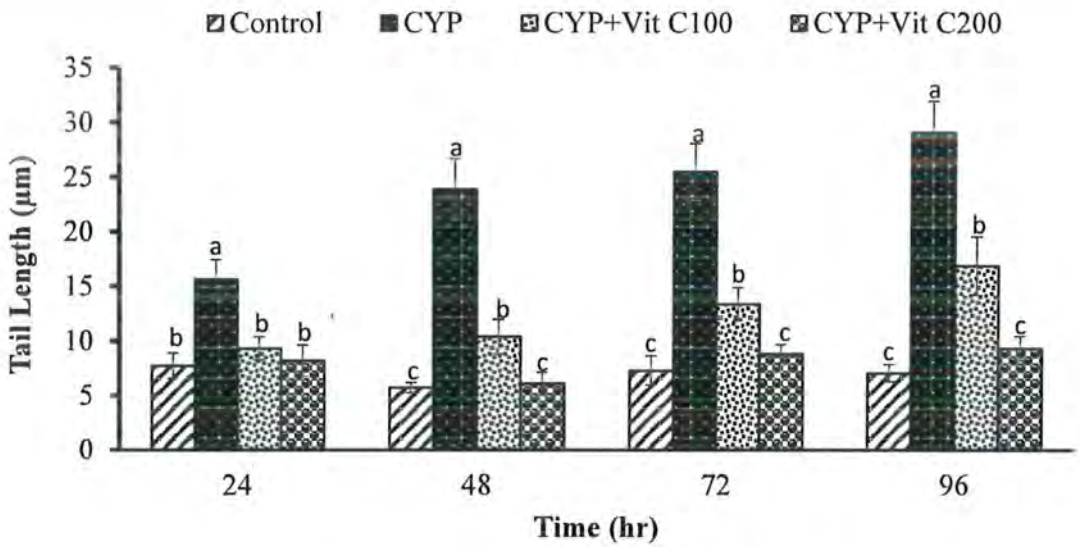

Fig 19: Tail length (µm) formed in peripheral erythrocytes of rohu at different time intervals after exposure of LC_{50} of CYP and ameliorative role of Vit C. Data are presented as mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

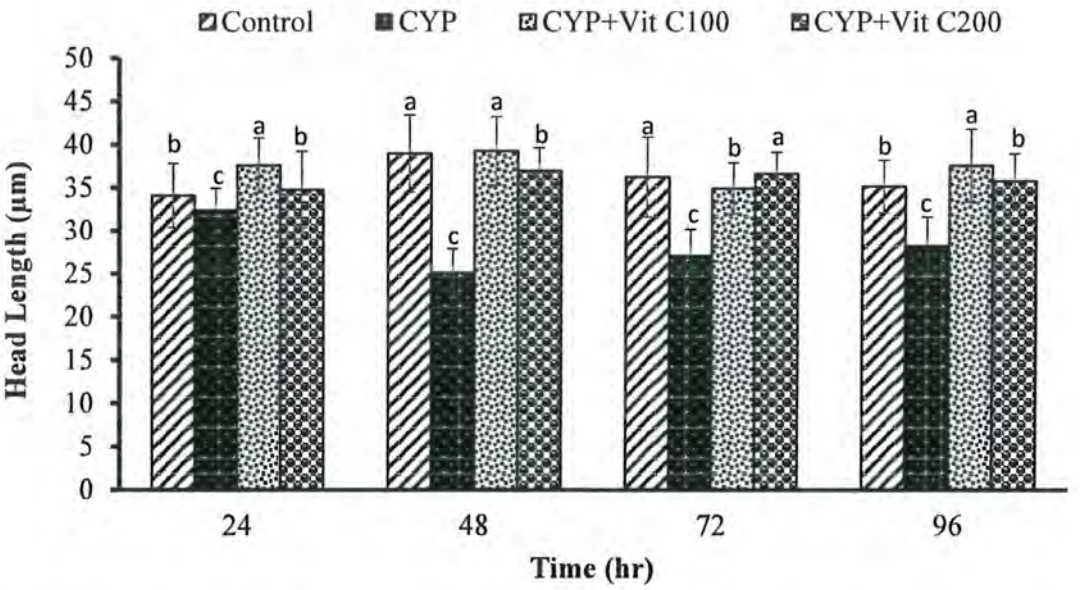

Fig 20: Head length (µm) of comets in peripheral erythrocytes of rohu at different time intervals after exposure of LC_{50} of CYP and ameliorative role of Vit C. Data are presented as mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

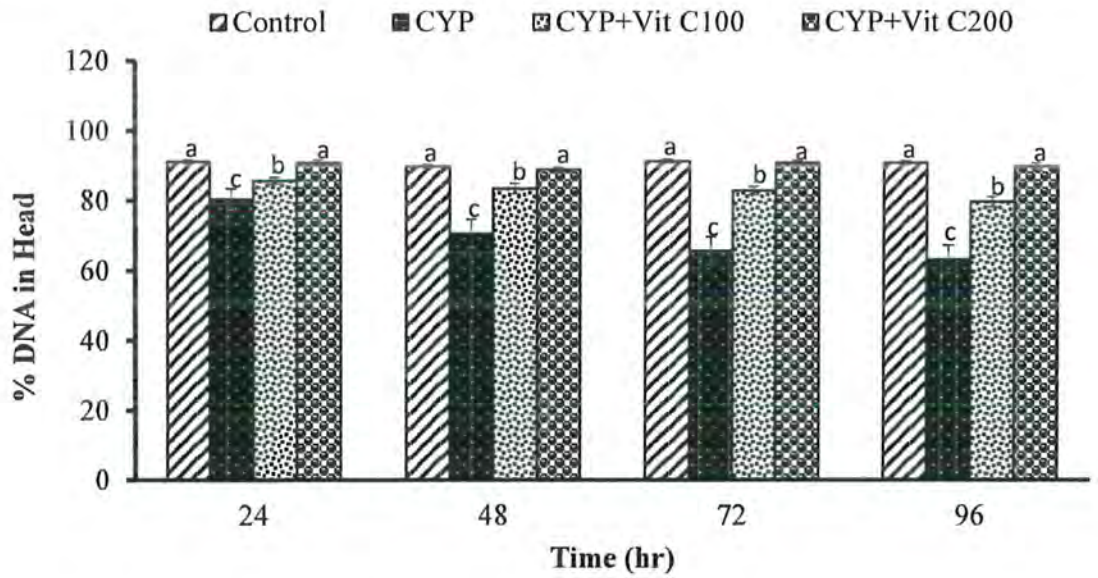

Fig 21: Percent DNA in head (%) of comets in peripheral erythrocytes of rohu at different time intervals after exposure of LC_{50} of CYP and ameliorative role of Vit C. Data are presented as mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

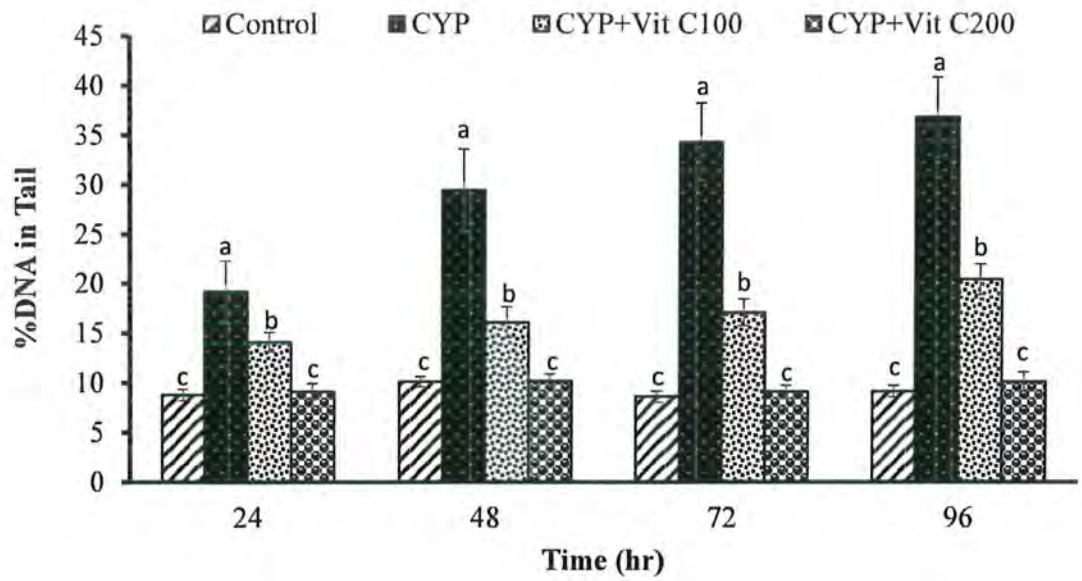

Fig 22: Percent DNA in tail (%) of comets in peripheral erythrocytes of rohu at different time intervals after exposure of LC_{50} of CYP and ameliorative role of Vit C. Data are presented as mean ± S.E. (n = 3). Means with different letters are significantly different ($P<0.05$).

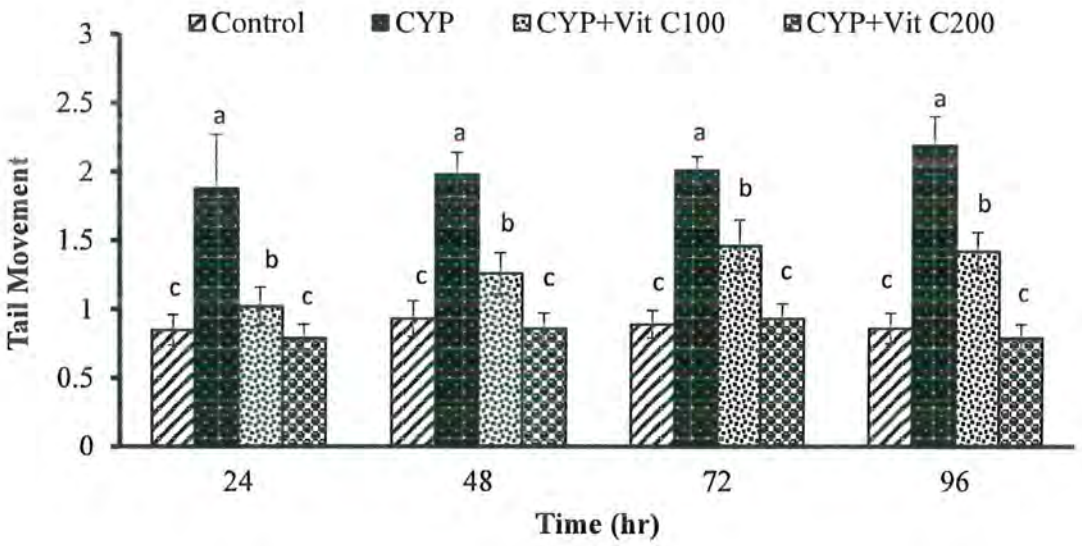

Fig 23: Tail movement of comets in peripheral erythrocytes of rohu at different time intervals after exposure of LC_{50} of CYP and ameliorative role of Vit C. Data are presented as mean ± S.E. (n = 3). Means with different letters are significantly different (P<0.05).

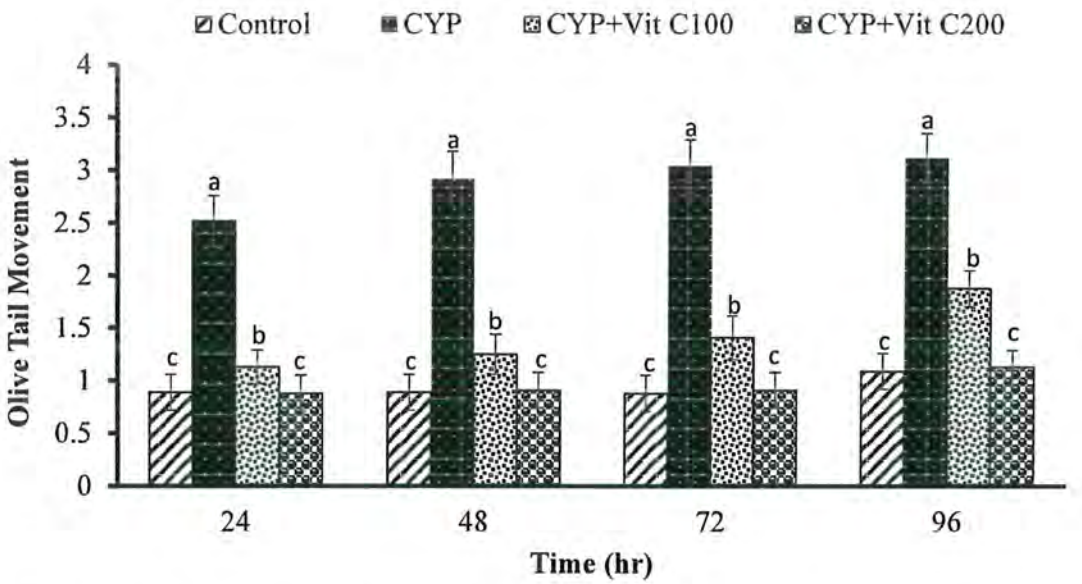

Fig 24: Olive tail movement of comets in peripheral erythrocytes of rohu at different time intervals after exposure of LC_{50} of CYP and ameliorative role of Vit C. Data are presented as mean ± S.E. (n = 3). Means with different letters are significantly different (P<0.05).

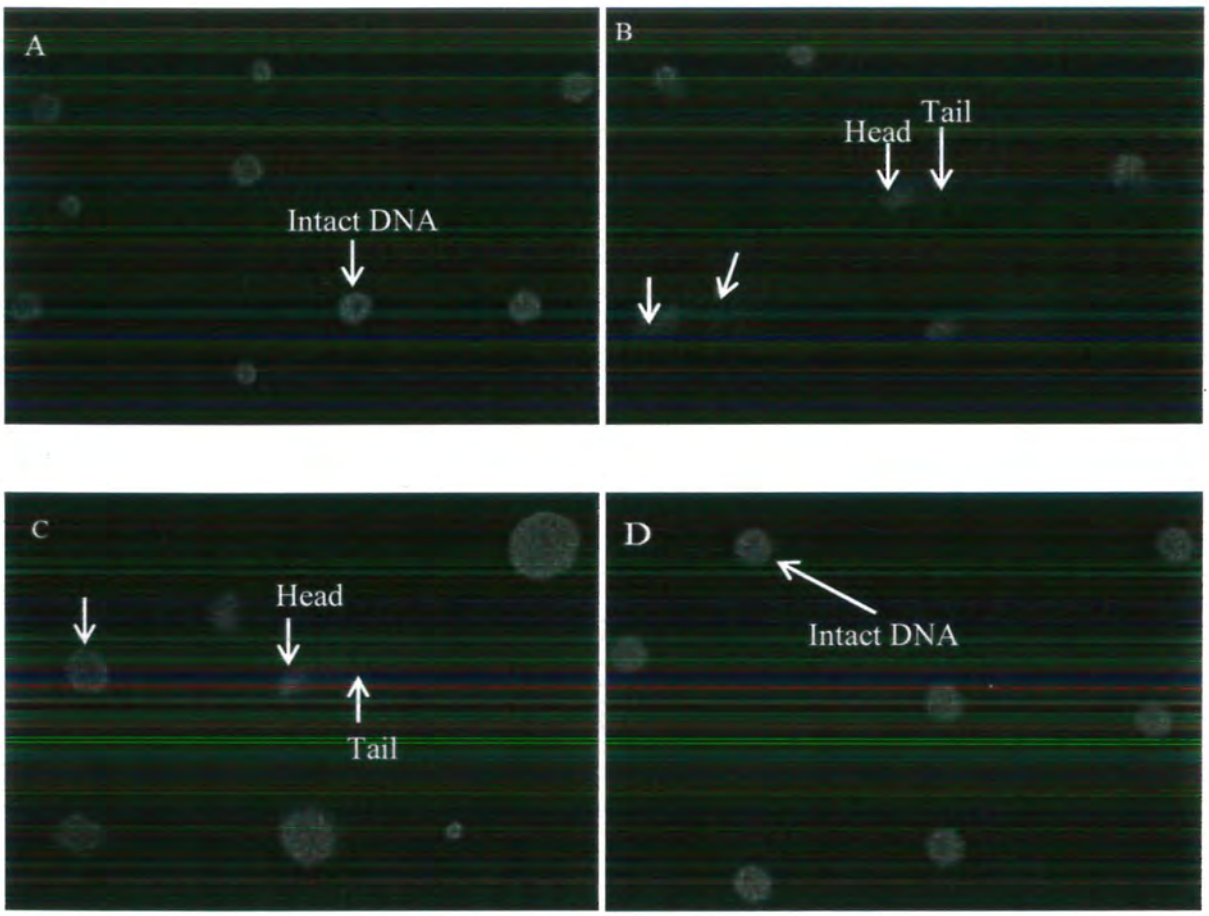

Fig 25: Fluorescent photomicrograph (40x) of peripheral blood erythrocytes after 96 hr of exposure of rohu to LC_{50} of CYP using comet assay (stain: Acridine orange). A) Intact DNA in Group 1st (Control). B) Comets with longer tail formed in Group 2nd (CYP alone). C) Comets with short tail and Intact DNA in Group 3rd (CYP+Vit C_{100} mg/kg diet). D) Intact DNA in rohu in Group 4th (CYP+Vit C_{200} mg/kg diet).

DISCUSSION

DISCUSSION

Toxicity Evaluation

In the current study the acute concentration of CYP causing 50% mortalities at 96 hr was found to be 4.5 µg/L for *Labeo rohita*. This value was greater than 4.0 µg/L reported by Marigoudar et al. (2009) for rohu but lower than 5.0 µg/L reported by Sridevi (1991) and 6.0 µg/L reported by Reddy and Bashamonideen (1989) for the same species. Somehow greater LC_{50} values of CYP was observed for different fish species such as 7.5 µg/L for rainbow trout (Coats and O.Donnel-Jeffery, 1979), 8.0 µg/L for rainbow trout (Chapman et al., 1981), 7.0 µg/L for *Salmo gairdeneri* (Edwards et al., 1986), 10.075 µg/L for *Cyprinus carpio* (Reddy, 1987), 9.00 µg/L for *Tilapia mossambica* (Reddy and Yellamma, 1991), 65.00 µg/L for *Cyprinus carpio* (Reddy et al., 1995), 5.13 µg/L for *Cirrhinus mrigala* (Prashanth and David, 2010) and 63.00 µg/L for *Tor putitora* (Ullah et al., 2014e). Changes in LC50 values for different fish species or even for the same species might be due the pesticides' formulation as well as stereochemistry of their molecules (FMC Corporation, 1989). In the present study and Marigoudar et al. (2009) used the same CYP type (PESTANAL®, analytical standard) but a little difference might be due to fish size, as they used smaller (2±0.2 g, 4±0.25 cm) fish as compared to the ones used in our study (6.55±1.01 g, 8.17±0.794 cm).

Pyrethroids' toxicity is also dependent on the stereochemistry of their molecules as isomers in pesticides' formulations vary in their precise toxicity. A pesticide using a single isomer base is relatively more toxic as compare to the one using various isomers combinations in their formulation (Bradbury and Coats, 1989). Beside this pesticide toxicity is also correlated on the carriers of inert ingredients, contaminants and active ingredients (FMC Corporation, 1989). Moreover toxicity of a chemical to fish is also dependent on various other factors such as size, age and health of the fish, and prevailing temperature (Abdul-Farah et al., 2004). Temperature observed in the present study was 25.8±1.2°C and no correlation between LC_{50} value and temperature was observed, but some previous existing studies suggested an inverse relation between temperature and LC_{50} of pesticides (Kumaragura and Beamish, 1981). According to Singh et al. (2010) pyrethroids are more toxic in winter as compare to summer season and observed approximately tenfold difference in LC50 values for 96 hr at 10, 15 and 20°C. Similarly an inverse relationship was observed concerning toxicity of pesticides and body weight of the fish (WHO, 1992).

Behavioral Toxicity

In the current study a time dependent behavioral response was observed after exposure of rohu to LC_{50} of CYP. While evaluating CYP toxicity for finding LC_{50}, it was observed that there was a direct relationship between CYP concentration and behavioral changes of rohu. CYP LC_{50} concentration led to different symptoms of poisoning such as fishes in Group 1st (Control, not exposed to CYP) and Group 4th (fish fed Vitamin C enriched diet at the rate of 200 mg/Kg diet) were active enough and well-coordinated. Fish in Group 2nd (fish fed basal diet) and Group 3rd (fish fed diet supplemented with vitamin C at the rate of 100 mg/Kg diet) showed time dependant behavioral responses like initially became hyper excited and irritable, then jumping, restlessness, mucus secretion, equilibrium loss and turning upside down were observed. With the passage of time, fish in Group 2nd and 3rd became lethargic with erratic movement. They were observed to gulp air with jerky globular movements and come to surface. These fish settled down with equilibrium loss, rolled their bodies and tremored before death. These fish also used to rise to the water surface for avoiding toxic surroundings. After death, gills of the deceased fish were turned brown from red. Fish in Group 1st and Group 4th did not show any of these changed behaviors indicating CYP stress account for these changes while vitamin C at the rate of 200 mg/Kg diet provided protection against CYP induced stress.

Similar alterations in behavioral responses were also observed in previous studies on pyrethroids toxicity in various species of fish such as *Oryzias latipes* (Rice et al., 1997), *Labeo rohita* (Marigoudar et al., 2009), *Cyprinus carpio* (Chebbi and David, 2009), *Silurus glanis* (Köprücü et al., 2006) and *Tor putitora* (Ullah et al., 2014e). These all investigators reported time and concentration dependent responses. Sandhu and Brar (2000) used high dose of CYP and reported coma due to respiratory depression, weakness and convulsions which eventually led to death. The very similar signs were observed during our study, but sampling of fish was carried out before mortality.

The abnormal behavioral responses observed and earlier reported might be due to pyrethroids' interaction with Ach (cholinergic neurotransmitter acetylcholine) (Chandra, 2008; Sharbidre et al., 2011). Usually in regular muscular function and behavior under no stress, ACh is released to synaptic cleft. After release AChE (acetylcholinesterase) enzyme hydrolyzes ACh and terminate synaptic transmission (Kopecka et al., 2004) but CYP caused neurotoxicity and inhibited action of AChE (Bibi et al., 2014). Thus, CYP presence leads to

ACh accumulation in nerve ending and consequently results in disturbance of nervous activities by over stimulation (Sharbidre et al., 2011). Eventually the prolonged muscle fibers stimulation causes paralysis and ultimately death (Purves et al., 2008). Moreover the time dependent abrupt fast swimming and lethargy due to muscle spasm might be associated with suffocation, respiratory dysfunction and neurological disruption of nerves' functions (Ullah et al., 2014e).

Protein Content

Results of the current study revealed a significant decrease of total protein content in brain, gills, liver and muscles of rohu after exposure to LC_{50} of CYP. The decrease was observed in a time dependent manner. Protein plays a vital role in the function and structure of the cells and occupies a key position in cell metabolism (Murray et al., 2007). The physiological activities of an animal are indicated by its proteins' metabolic status (Nelson et al., 2005). There is a rich literature documenting changes in protein content in fish tissues after exposure to different pesticides (Ahmad et al., 2012). A significant decrease was observed in total protein content of *Colisa fasciatus* after exposure to 40 and 60%, 24 hr LC_{50} CYP (Singh et al., 2010). A similar time dependent decrease was observed in *Danio rerio* after exposure to λ-cyhalothrin by Ahmad et al. (2012). Dimethoate and carbaryl pesticide also caused time dependent reduction in total protein contents in muscle and liver of *Lymnaea acuminate* (Tripathi and Singh, 2003). The current study also revealed time dependent decline in protein contents in brain, gills, liver and muscle of *Labeo rohita* after exposure to LC_{50} of CYP at different time intervals for 96 hr. Higher protein inhibition was observed in liver. Reduction in total protein contents might be due to low feeding under stress condition. It might also be due to protein catabolism or other metabolic processes for fulfilling energy demand. According to Mommsen and Walsh (1992), fish gets their energy by catabolizing protein under stress. David et al. (2005) also observed similar trend of protein decline in *Cyprinus carpio* after exposure to CYP.

Some other studies also reported protein depletion in fish tissues after exposure to different pesticides such as *Channa striatus* (Tantarpale, 2011) and *Oreochromis niloticus* (Korkmaz et al., 2009) exposed to CYP and *Labeo rohita* exposed to deltamethrin (Neeraja and Giridhar, 2014). According to Ray and Banerjee (1998) pesticides stress influence tissue protein conversion into soluble fractions that use to move in blood for utilization.

Lipid Peroxidation

In the current study a time dependent increase in lipid peroxidation (LPO) was observed after exposure to LC_{50} of CYP, indicated by time course increase in TBARS level. The inability of an animal's defense system to completely neutralize reactive oxygen species (ROS), leads to oxidative damage of membrane lipids by ROS (Ahmad et al., 2000b). Therefore LPO is considered as a key outcome of oxidative stress (Hermes Lima, 2004). Like current result, many other studies reported LPO induction after pesticides exposure. Bibi (2011) observed increase MDA level in *Cyprinus carpio* whereas Parthasarathy and Joseph observed increase LPO in *Oreochromis mossambicus* after exposure to γ- cyhalothrin and CYP respectively. Atif et al. (2005) and Köprücü et al. (2008) observed an elevated level of MDA in *Channa punctatus* and *Unio elongatulus euchres* respectively after deltamethrin exposure. Similarly other investigators observed increase level of LPO in other fish species such as *Poecilia reticulate* (Sharbidre et al., 2011) and *Clarias gariepinus* (Adeyemi et al., 2013) after exposure to Diazinon and CYP respectively.

LPO is a complex process. It displays higher activity in membranes richer in polyunsaturated fatty acids. It causes oxidative damage to specific tissues (Adeyemi et al., 2013) by destroying its membranous structure by decomposing double bond present in unsaturated fatty acids (El-Beltagi and Mohamed, 2013). The LPO level in rohu after exposure to CYP showed time dependent and tissue specific pattern as LPO induction was higher in liver followed by brain. The result of the current study is in accordance to the previous studies where CYP induced higher LPO in liver in *Clarias gariepinus* (Adeyemi et al., 2013) and *Tor putitora* (Ullah et al., 2014e) while in liver of *Channa punctatus* (Kaur et al., 2011) after exposure to Deltamethrin. Highest lipid peroxidation activity in liver might be due to numerous redox cycling and excessive ROS production in liver (Reinke, 1995).

Antioxidant Enzymes

The present study revealed induction of antioxidative stress in rohu after exposure to CYP in time dependent manner, which is in accordance with earlier studies on *Labeo rohita* (Marigoudar et al., 2012; Sivaperumal and Sankar, 2011), *Lepomis macrochirus* (Elia et al., 2002) *Cyprinus carpio* (Bibi, 2011), *Geophagus brasiliensis* (Filho et al., 2001), *Prochilodus lineatus* (Langiano and Martinez, 2008) and *Tor putitora* (Atika, 2011; Ullah et al., 2014e). Pyrethroids are rapidly absorbed and efficiently distributed in various organs of fish due to

their disposal mechanisms (Mahaboob et al., 2005). The current results clearly indicated the failure of antioxidant defense system of *Labeo rohita* against oxidative stress induced by CYP. This might also be the reason of imbalance in cellular oxidative status due to oxidative damage. Previous studies correlated oxidative stress with pyrethroids induced toxicity (Kale et al., 1999; Giray et al., 2001), though studies concerning in vivo susceptibility of fish to CYP nducedoxidative stress by are limited.

Increase in antioxidant activity may be due to the presence of xenobiotics, which suggest adaptation of organism to the appeared condition (Doyotte et al., 1997). Different fish species showed different response level to various toxicants which might be due to variation in their antioxidant potentiality (Ahmad et al., 2000a). The current study revealed that oxidative damage may be associated with the subcellular effect of CYP in fish as previous studies showed the putative role of CYP in generating free radicals (Altuntas et al., 2002). CYP has also been concluded as a degrading agent for renal cells and hepatocytes (Sohn et al., 2004). Higher elevation in antioxidant activities was observed in liver of rohu, suggesting liver as a central organ in detoxification processes thus facing maximum xenobiotic exposure as well as its metabolites (Marigoudar, 2012). Antioxidant enzymes activities (AOA) are considered as an alarming sign for the presence of toxicant or environmental pollutant (Rosety et al., 2005). There is a rich literature documenting pyrethroids as potent inducer of oxidative stress and modulators of antioxidant system of fish (Üner et al., 2001; Sankar et al., 2012). In antioxidant enzymes system, CAT/SOD systems provide a first line of defense against ROS. CAT is responsible for the detoxification of H_2O_2 to water while SOD help to dismutase superoxide radical O_2^- to hydrogen peroxide (H_2O_2). Glutathione peroxidase (GSH-Px) reduces organic peroxides through glutathione depending reaction and H_2O_2. Glutathione reductases (GR) catalyze NADPH depending regeneration of glutathione for oxidized glutathione produced by glutathione peroxidase.

Elevation of catalase activity was observed in all the four studied tissues of rohu, *L. rohita*. Catalase enzyme has a vital role in detoxifying toxicants and its metabolites. Higher CAT activity in liver suggested its major role in detoxification of CYP as concluded by Akhtar et al. (1994) and Sayeed et al. (2003). The current study thus suggested detoxification of CYP and its metabolites in liver, which might be mainly due to its exposure route and characters. According to Edwards and Millburn (1985) main route for CYP detoxification is hydroxylation and is removed as conjugates of glucuronide. It was observed that liver showed

stronger against oxidative stress as compare to other tissues which might be due to the fact that it is main spot for generation of maximum free radicals and several oxidative reactions (Avci et al., 2005; Gül, et. al, 2004). Furthermore increase in CAT activity observed in the present study might be due to slow eliminating process of CYP from the studied organs. Elevation in CAT activities in response to pesticides was also observed in previous studies conducted on *Tor putitora* (Atika, 2011; Ullah et al., 2014e), *Cyprinus carpio* (Bibi, 2011), *Prochilodus lineatus* (Langiano and Martinez, 2008), *Lepomis macrochirus* (Elia et al., 2002) and *Geophagus brasiliensis* (Filho et al., 2001).

Increase in glutathione reductase (GR) activity in the current study is in accordance with many other studies conducted in the past (Ullah et al., 2014e; Marigoudar, 2012). Elevation of GR activity might be due to the ameliorative role of GR pathway in different fish organs that proficiently regulates ROS formation under oxidative stress. This compensate response of GR and other enzymes including POD, SOD and CAT in fish, clearly prevent free radicals and byproducts accumulation in stressed condition (Ullah et al., 2014e). It is well documented that pesticides or herbicides are transformed into their respective metabolites in water. These are having higher or similar toxicity as compare to their parental product (Tchounwou et al., 2000).

In the current study the time dependent induction of Glutathione peroxidase (GSH-Px) activity does demonstrate a possible defense provided against the increased LPO levels at different time intervals after the exposure of rohu to LC_{50} of CYP. GSH-Px is mainly involved in removing hydrogen peroxides and organic compounds, consequently plays a key role against LPO. It is considered to be very much important for protection of membranes against LPO induced damage, suggesting the key detoxifying function of GSH-Px in terminating the propagation of radical chain (Oost et al., 2003). The result of the current study is in accordance with the previous studies on *Oreochromis niloticus* (Almeida et al., 2002), *Channa punctatus* (Sayeed et al., 2003) and *Carassius auratus* (Zhang et al., 2004).

The considerable increase in total reduced glutathione (GSH) level in all tissues at different time intervals after CYP exposure might be due to supplying an increase level of GSH, leading to oxidized glutathione formation as well as efficient regeneration of GSH. GSH detoxify electrophiles and prevents cellular oxidative stress (Sies, 1999; Hasspieler et al., 1994). During oxidative stress GSH level may elevate as a result of increased synthesis. For removing xenobiotics through direct conjugations either with glutathione or through

glutathione-S-transferase, GSH level get decrease. Therefore increase in GSH level might be for fulfilling the demand of the antioxidant defense system. Furthermore the steady increase in GSH level might also be an indication of exhaustion in biotransformation (phase 2nd), which is also established by elevation in glutathione-s-transferase activity at different time interval in brain, gills, liver and muscles after exposure to acute concentration of CYP (Sivaperumal, 2008).

Time dependent increase in Glutathione-S-transferase (GST) activity was also observed in all the tissues at different time intervals which might be related to developing defensive mechanism for counteracting CYP induced effects. GST is responsible for detoxifying and excreting xenobiotics as well as their metabolic products (Jokanovic, 2001). GST got elevated during the current study as it plays the key role of tissue protection from oxidative stress (Banerjee et al., 1999; Fournier et al., 1992) and removes highly reactive electrophiles before binding with nucleophilic compounds in tissues (Sivaperumal, 2008). Increase in GST activity in the present study is in accordance with the study conducted on freshwater characid fish (FoMonterio et al., 2006) and *Labeo rohita* (Sivaperumal and Sankar, 2011).

Enzymes of Protein Metabolism

The significant elevation of GDH, ALT, AST and LDH in the studied tissues after CYP exposure revealed greater relation of oligomers of these enzymes to stress. The results also indicated contribution of oxidative deamination to higher ammonia production. Ammonia in greater amount is produced which is not eliminated but are released through the activity of glutamate dehydrogenase. This is then utilized for the synthesis of amino acid via transaminases (Prashant, 2003). Increase in GDH in all tissues suggested the probability of needing α-ketoglutarate in Krebs cycle for energy liberation. This in turn depicts an increasing requirement for α-ketoglutarate. The regulatory role of GDH in deamination processes is documented from previous studies (Shobha et al., 2001; Reddy and Yellama, 1991). GDH plays a basic role in biosynthesis and catabolism of amino acids and can catalyze oxidative deamination in reversible reaction such as transforming glutamate to ammonia and α-ketoglutarate (Begum and Vijayaraghavan, 1998). Thus, the increased GDH activity demonstrated elevated glutamate deamination and ammonia formation (Prashanth and Neelagund, 2008).

Increase activities of ALT and AST observed in the present study might be due to active transamination of amino acids. This is for providing keto acid in the citric acid cycle (Al-Ghanim, 2014). Increase activities of AST and ALT might be on account of production of these enzymes due to CYP induced stress. This elevation might be helpful to fish for structural reorganization of proteins and incorporating keto acid into citric acid cycle in order to facilitate gluconeogenesis or production of energy. Elevation in transaminases may also be associated with urea formation (Ramna and Ramamurthi, 1983).

The observed time dependant increase in LDH activity in the present study might be due to necrosis and subsequent leakage into blood stream (El-Sayed and Saad, 2008). The same results in LDH activity was observed by Kumar et al. (2012) in *Channa punctatus* after exposure to CYP and λ-cyhalothrin. The steady rise in the activities of these enzymes might lead to metabolic compensation and allowing fish to acclimate to the induced stress conditions or may also be linked with elimination of ammonia of the body by producing more glutamate (Prashanth and Neelagund, 2008).

Digestive Enzymes

In the present study increase in the activities of amylase, cellulase and protease was observed at different time intervals after exposure to LC_{50} of CYP. Increase in the activity of amylase was also observed in *Cyprinus carpio* after exposure to two different pesticides, Terbutryn and Simazine (Velisek et al., 2011, 2012). Increase in the activities of protease and cellulase was also observed by many researchers such as Reddy and Bashamohideen (1995), Smet and Blust (2001), David et al. (2005) and Manjunatha et al. (2014) etc. Increase in the activities of digestive enzymes might be due to increase demand for higher level energy requirement and low feeding activity in response to CYP stress. It might also be due to increase in catabolic activities in order to provide as much enery as required to fish under stress condition.

DNA Damage Induction

The result of the current study revealed induced DNA damage in blood erythrocytes of rohu after exposure to LC_{50} of CYP. The DNA damage occurred was in the form of number of comets observed per 120 cells, comet length, tail length and head length, % DNA in head, % in tail, tail movement and olive tail movement. The results of the current study clearly demonstrated the genotoxic effect of CYP in rohu by inducing a significant increase

in number of comets, % DNA in tail, tail length and olive tail movement in the group fed with basal diet (Group 2nd) followed by the group fed basal diet supplement with vitamin C at the rate of 100 mg/Kg diet. No significant difference observed in fish of Group 1st (Control) and fish of Group 4th fed vitamin C enrich diet at the rate of 200 mg/Kg diet suggest the strong antioxidant potential of vitamin C against ROS, which is mainly responsible for disturbing biochemical parameters of fish (Sies, 1986, 1988; Anderson *et al.*, 1994; Ali *et al.*, 2015). DNA is of the most vulnerable among these biomolecules that undergo severe damage in a shorter time period (Halliwell and Arouma, 1991).

DNA damage has been observed in a number of mammalian cells after induction of oxidative stress, reviewed in detail by Halliwell and Arouma (1991). In vertebrate, including fish DNA damage involves the conversion of double stranded DNA to single strand or chromosomal aberrations and it can be measured by using comet assay (Cortés-Gutiérrez et al., 2014) or Fluorimetric method (Bhusate et al., 1992). Schraufstätter et al. (1988) reported the direct relationship between ROS, ROS generating enzymes and DNA damage while other indicating the relation of ROS like O_2^-, H_2O_2, HOCl and O_3 with induction of DNA damage (Arouma et al., 1989; Brawn and Fridovich, 1981; Lesko et al., 1980; Rowley and Halliwell, 1983).

Moreover, Mello Filho et al.(1984) discussed the possibility of the involvement of H_2O_2 in DNA damage to. H_2O_2 can cross membranes easily, thus penetrating nucleus and reacts with iron or copper ions present in chromosome (Prutz et al., 1990) and promote H_2O2 dependent damage to isolated DNA (Arouma et al., 1991; Sagripanti and Kraemer, 1989) and DNA within chromatin (Dizdaroglu et al., 1991) by forming OH (Halliwell and Arouma, 1991), which is highly reactive and unable to diffuse significant distances within the cells. DNA damage might also be due the fact that metal ions released within the cell as a result of oxidative stress bind to the DNA (Halliwell, 1987). Thus, just as oxidative stress causes rise in intracellular free Ca^{2+}, it might cause a rise in intracellular free iron and/or copper ions that could bind to DNA and make it a target for oxidative damage (Halliwell, 1987; Sakaida et al., 1990).

The genotoxicity observed in current study confirmed the previous conducted studies on DNA damage induction in various fish species in response to different toxicants such as *Clarias gariepinus* (Farombi et al., 2007), *Carrasius auratus* (Toyoizumi *et al.*, 2008), *Hyphssobrycon luetkenii* (Scalon et al., 2010), *Oreochromis mossabicus* (Ahmed et al.,

2011), *Channa punctatus* (Kumar et al., 2013) and by Kousar and Javed (2015) in *Labeo rohita*, *Ctenopharyngodon idella, Catla catla* and *Cirrhina mrigala.*

Protective role of Vitamin C

Highest level of oxidative stress resulted in low protein content, higher antioxidative enzymes' activities, increase in protein metabolic enzymes' activities and DNA damage was observed in fish fed with basal protein diet (Group 2nd) followed by fish fed supplemented with vitamin C at the rate of 100 mg/Kg diet (Group 3rd). No significant different observed in Group 1st (Control, not exposed to CYP) and Group 4th (Fish fed with Vitamin C at the rate of 200 mg/Kg diet) suggesting strong ameliorative role of Vitamin C against CYP induced biochemical toxicity in rohu at the rate of 200 mg/kg diet but did not provide protective role at the rate of 100 mg/kg diet. This might be due to the strong antioxidant potential of Vitamin C boosting the antioxidant defense system of fish and immunity. Thus the present studies confirm the previous conducted studies on ameliorative, modulating, immunizing and protective role of vitamin C in different fish species such as *Salmo salar* (Hardie et al., 1991; Thompson et al., 1993) *Sparus aurata* (Ortuno et al., 1999, 2001; Cuesta et al., 2002), *Labeo rohita* (Nayak et al., 2007), *Heteropneustes fossilis* (Saha and Kaviraj, 2012), and *Clarias batrachus* (Narra et al., 2015). DNA damage is associated with oxidative stress, therefore protecting from oxidative stress resulted DNA protection against CYP. Sram et al. (2012) comprehensively reviewed the protective role of vitamin C in DNA damage prevention in different species.

It is well established from various previous studies that DNA damage is directly correlated with ROS and elevated level of LPO (Walz et al., 2000; Freitas et al., 2004). Vitamin C has many non-enzymatic actions and is a powerful water-soluble antioxidant. It protects low density lipoprotein from oxidation and reduces harmful oxidant (Santos et al., 2009). Due to higher integrity of lipoprotein, H_2O_2 cannot easily penetrate biological membranes, thus cannot approach nucleus and consequently cannot react with free copper or iron ions (Halliwell and Arouma, 1991). This inhibits OH formation which reacts with metal ions bound upon or very close to the DNA, thus resulting in DNA damage (Halliwell and Gutteridge, 1989). The effective role of Vitamin C against ROS degradation and protector as a strong antioxidant is well established (Jakeman and Maxwell, 1993). Moreover, Sram et al. (2012) also comprehensively reviewed the protective role of vitamin C in DNA damage prevention in terms of chromosomal aberration, damage induced in micronuclei, and DNA

strand breakage. Thus, it appears that in present study, CYP induced oxidative stress and generation of ROS while vitamin C provides protection against the deleterious effects CYP by inhibiting the formation of ROS. Therefore, in the current study vitamin C might have protected DNA from oxidative stress due to CYP exposure.

Conclusion

The present study showed that CYP is distinctly toxic to rohu. Moreover, locality based species should be selected for toxicological studies because expressions or responses to toxicants vary from species to species and condition to condition. This research also warrants a vital need to assess further eco-toxicological studies for gathering wider ranges of data regarding fish and other animals as well to know about the far-reaching spectrum of CYP as well as other available pesticides. This will not only help to establish permissible limits and safer level for different toxic chemicals for bio-organisms but also to protect imbalance of residues in aquatic ecological cycle. This type of study might be very handy to make comparison of various aquatic species regarding their sensitivity as well as influence of different chemicals by finding their LC_{50} values which will help to derive safer and environment friendly concentrations.

Moreover results of the present study revealed that oxidative stress contribute to CYP induced biochemical toxicity in term of protein content, protein metabolic enzymes and DNA damage. The current results are indicating aquatic pollution load of CYP in effected rohu populations, so it might provide help in pollution diagnosis. Our results also indicated the modulating role of vitamin C in indigenous culturable species rohu *Labeo rohita* at the rate of 200 mg/Kg diet but not at the rate of 100 mg/Kg diet. It was observed that vitamin C can compensate the consequences of CYP induced toxicity to biochemical parameters including protein, antioxidant enzymes, protein metabolic enzymes and DNA in rohu, *Labeo rohita*.

REFERENCES

REFERENCES

Abdul–Farah, M., Ateeq, B., Ali, M.N., Ahmad, W., 2004. Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. Chemosphere 55, 257–265.

Abu-Darwish, M.S., Al-Fraihat, A.H., Al-Dalain, S.Y.A., Afifi, F.U., Al-Tabbal, J.A., 2011. Determination of essential oils and heavy metals accumulation in *Salvia officinalis* cultivated in three intra-raw spacing in ash-shoubak, Jordan. Int. J. Agric. Biol. 13(6), 981-985.

Adeyemi, J.A., Atere, T.G. and Deaton, L.E., 2013. Oxidative damage and changes in Glutathione S-transferase activity in juvenile African catfish, *Clarias gariepinus* exposed to cypermethrin and chlorpyrifos. Biokemistri 25, 113-117.

Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C.T., Ayyappan, S., 2004. Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). Ecotoxicol. Environ. Saf. 58(2), 220-226.

Agarwal, S.K., 2002. Pollution Management – Water Pollution, A.P.H. Publishing Corporation, New Delhi, p. 1-2.

Ahmad, I., Hamid, T., Fatima, M., Chand, H.S., Jain, S.K., Athar, M., Raisuddin, S., 2000a. Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) as a biomarker of paper mill effluent exposure. Biochim. Biophys. Acta 1519, 37-48.

Ahmad, M.K., Sharma, D.K., Ansari, S., Ansari, B.A., 2012. Effect of lambda–cyhalothrin and Neem gold on some biochemical parameters in the gill, liver, and ovary of zebra fish, *Danio rerio* (Cyprinidae) Arch. Pol. Fish. 20, 19–25.

Ahmad, R.S., Seth, V., Pasha, S.T., Banerjee, B.D., 2000b. Influence of dietary ginger (*Zingiber officinales* Rosc) on oxidative stress induced by malathion in rats. Food Chem. Toxicol. 38, 443-450.

Ahmed, M.K., Habibullah-Al-Mamun, M., Hossain, M.A., Arif, M., Parvin, E., Akter, M.S., Khan, M.S., Islam, M.M., 2011. Assessing the genotoxic potentials of arsenic in tilapia (*Oreochromis mossambicus*) using alkaline comet assay and micronucleus test. Chemosphere 84, 143-149.

Ai, Q., Mai, K., Zhang, C., Xu, W., Duan, Q., Tan, B., Liufu, Z., 2004. Effects of dietary vitamin C on growth and immune response of Japanese seabass, *Lateolabrax japonicus*. Aquaculture 242, 489-500.

Akhtar, M.H., Mahadevan, S., Paquet, A., 1994. Comparative metabolism of deltamethrin and 3-phenoxybenzoic acid in chickens. J. Environ. Sci. Health 29, 369-394.

Akinrotimi, O.A., Gabriel, U.U., Ariweriokuma, S.V., 2012. Haematotoxicity of Cypermethrin to African Catfish *Clarias Gariepinus* under Laboratory Conditions. J. Environ. Eng. Technol. 1(2), 20-25.

Al-Ghanim, K.A., 2014. Effect of a Synthetic Pyrethyroid, Cypermethrin, on Aminotransferases and Glutamate Dehydrogenase Activities in Gill, Liver and Muscles of a Freshwater Fish, *Cyprinus carpio*. Pak. J. Zool. 46(4), 997-1001.

Ali, D., Kumar, P.G., Kumar, S., Ahmed, M., 2015. Evaluation of genotoxic and oxidative stress response to dimethoate in freshwater fish *Channa punctatus* (Bloch). Chem. Spec. Bioavail. 26(2), 111-118.

Ali, J., Ullah, N., Khan, F.A., Rahman, Z., Ahmad, I., Hassan, S., Ahmad, I., 2013. Bacteriological quality analysis of drinking water of rural areas of Peshawar, Pakistan. Am-Eur. J. Agri. Environ. Sci. 13(9), 1202-1206.

Almeida, J.A., Diniz, Y.S., Marques, S.F.G., Faine, L.A., Ribas, B.O., Burneiko, R.C., Novelli, E.L., 2002. The use of oxidative stress responses as biomarkers in Nile tilapia (*Oreochromic niloticus*) exoposed to in vivo cadmium contamination. Environ. Int. 27(8), 673-679.

Altuntas, I., Delibas, N., Sutcu, R., 2002. The effects of organophosphate insecticide methidathion on lipid peroxidation and anti-oxidant enzymes in rat erythrocytes: role of vitamins E and C. Hum. Exp. Toxicol. 21(12), 681-685.

Anderson, D., Yu, T.W., Phillips, B.J., Schmerzer, P., 1994. The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the comet assay. Mut. Res. 307, 261-271.

Aprile, F.M., Bouvy, M., 2008. Distribution and enrichment of heavy metals in sediments at the Tapacura river basin, Notheastern Brazil. Braz. J. Aquatic Sci. Technol. 12(1), 1-8.

Areekijseree, M., Engkagul, A., Kovitvadhi, U., Thongpan, A., Mingmuang, M., Pakkong, P., Rungruangsak-Torrissen, K., 2004. Temperature and pH characteristics of amylase and proteinases of adult freshwater pearl mussel, *Hyriopsis bialatus* Simpson 1900. Aquaculture 234, 575–587

Arimoro, F.O., Ikomi, R.B., 2008. Response of Macroinverted rate communities to abattoir wastes and other anthropogenic activities in a municipal stream in the Niger Delta, Nigeria. Environmentalist 28, 85-98

Arouma, O.I., Halliwell, B., Gajewski, E., Dizdaroglu, M., 1989. Damage to the bases in DNA induced by hydrogen peroxide and ferric ion chelates. J. Biol. Chem. 264, 20509-20512.

Arouma, O.I., Halliwell, B., Gajewski, E., Dizdaroglu, M., 1991. Copper-ion-dependent damage to the bases in DNA in the presence of hydrogen peroxide. Biochem. J. 273, 601–604

Atamanalp, M., Yanik, T., Haliloglu, H.I., Aras, M.S., 2002. Alterations in the hematological parameters of rainbow trout, *Oncorhynchus mykiss*, exposed to Cypermethrin. Israeli J. Aquacult- Bamidgeh 54(3), 99-103.

Athanassopoulou, F., Pappas, I.S., Bitchava, K., 2009. An overview of the treatments for parasitic disease in Mediterranean aquaculture. Options Méditerranéennes 86, 65-83.

Atif, F., Parvez, S., Pandey, S., Ali, M., Kaur, M., Rehman, H., Khan, H.A., Raisuddin, S., 2005. Modulatory effect of Cadmium exposure on Deltamethrin-induced oxidative stress in *Channa punctatus*. Arch. Environ. Contam. Toxicol. 49, 371-377.

Atika, B., 2011. Effect of Selenium on Cypermethrin Induced Oxidative Stress in Mahseer (*Tor putitora*). M.Phil Thesis, Department of Animal Sciences, Quaid-i-Azam University Islamabad, Pakistan.

Avci, A., Kaçmaz, M., Durak, I., 2005. Peroxidation in muscle and liver tissues from fish in a contaminated river due to a petroleum refinery industry. Ecotoxicol. Environ. Saf. 6, 101–105.

Ayoola, S.O., Ajani, E.K., 2008. Histopathological effects of Cypermethrin on Juvenile African Catfish (*Clarias gariepinus*). World J. Biol. Res. 1(2), 1-14.

Banerjee, B.D., Seth, V., Ahmed, R.S., 2001. Pesticide-induced oxidative stress: perspectives and trends. Rev. Environ. Health 16, 1–40.

Banerjee, B.D., Seth, V., Bhattacharya, A., Pasha, S.T., Chakraborty, A.K., 1999. Biochemical effects of some pesticides on lipid peroxidation and free radical scavengers. Toxicol. Lett. 107(1-3), 33-47.

Begum, G., 2005. In vivo biochemical changes in liver and gill of *Clarias batrachus* during cypermethrin exposure and following cessation of exposure. Pest. Biochem. Physiol. 82(3), 185–196.

Begum, G., 2007. Cypermethrin-induced biochemical pertubations in freshwater fish *Clarias batrachus* at sublethal exposure and after released into freshwater. Drug Chem. Toxicol. 30(1), 55-65.

Begum, G., 2009. Enzymes as biomarkers of cypermethrin toxicity: response of *Clarias batrachus* tissues ATPase and glycogen phosphorylase as a function of exposure and recovery at sublethal level. Toxicol. Mechan. Meth. 19(1), 29–39.

Begum, G., Vijayaraghavan, S., 1998. Toxicity of rogor on total free amino acids and glutamate dehydrogenase in hepatic and muscle tissues of a fish. Ecol. Environ. Conserv. 4, 235-238.

Bergmeyer, H.U.C., 1965. Aminotransferases and related enzymes. In: Bergmeyer, H.U.C., Bernt, E., eds. Methods of Enzymatic Analysis. Vol. II. New York: Academic Press. pp. 735-739, 760-764.

Bernfeld, P., 1955. Amylase α and β, In Methods in Enzymology (Colowick SP, Kaplan NO, ed.), Academic Press Inc, New York 1, 149-158.

Bhusate, L.L., Herbert, K.E., Scott, D.L., Perrett, D., 1991. Increased DNA strand breaks in mononuclear cells from patients with rheumatoid arthritis. Ann. Rheum. Dis. 51, 8-12.

Bibi, N., 2011. Sublethal effect of locally used pesticide, karate on Acetylcholinesterase activity and antioxidant enzymes in fry of freshwater Cyprinid *Cyprinus carpio*. M.Phil Thesis, Department of Animal Sciences, Quaid-i-Azam University Islamabad, Paksitan.

Bibi, N., Zuberi, A., Naeem, M., Ullah, I., Sarwar, H., Atika, B., 2014. Evaluation of acute toxicity of karate and its sub-lethal effects on protein and acetylcholinestrase activity in Cyprinus carpio. Int. J. Agric. Biol. 16: 731-737.

Borges, L., Scotti, V., Siqueira, D.R., 2007. Changes in hematological and serum biochemical values in jundi'a *Rhamdia quelen* due to sub-lethal toxicity of Cypermethrin. Chemosphere 69(6), 920–926.

Bradbury, S.P., Coats J.R, 1989. Comparative toxicology of the pyrethroid insecticides. Rev. Environ. Contam. Toxicol. 108, 133–177.

Brawn, K., Fridovich, L., 1981. DNA strand scission by enzymically-generated oxygen radicals. Arch. Biochem. Biophys. 206, 414-419.

Carlberg, I., Mannervik, E. B., 1975. Glutathione level in rat brain. J. Biol. Chem. 250, 4475 -4480.

Carriquiriborde, P., Diaz, J., Mugni, H., Bonetto, C., Ronco, A.E., 2007. Impact of cypermethrin on stream fish populations under field-use in biotech-soybean production. Chemosphere 68, 613–621.

Casida, J.E., Gammon, D.W., Glickman, A.H., Lawrence, L.J., 1983. Mechanism of pyrethroid insecticides. An. Rev. Pharmacol. Toxicol. 23, 413–418.

Celik, A., Mazmanci, B., Camlica, Y., Askin, A., C¨omelekoglu, U., 2003. Cytogenitic effects of lambda-cyhalothrin on winstar rat bone marrow. Mutat. Res. 539, 91–97.

Celik, A., Mazmanci, B., Camlica, Y., C¨omelekoglu, U., Askin, A., 2005. Evaluation of cytogenetic effects of lambda-cyhalothrin on Wistar rat bone marrow by gavage administration. Ecotoxicol. Environ. Saf. 61, 128–133.

Chance, B., Maehly, A. C., 1955. Assay of catalase and peroxides. Methods Enzymol. 11, 764- 775.

Chandra, S., 2008. Toxic effect of Malathion on acetylcholinesterase activity of liver, brain and gills of freshwater catfish *Heteropneutes fossilis*. Environ. Conserv. 9, 45-52.

Chapman, R.A., Tu, C.M., Harris, C.R., Cole, C., 1981. Persistence of five pyrethroid insecticides on Rainbow trout. Bull. Environ. Contam. Toxicol. 26, 513-519.

Chebbi, S.G., David, M., 2009. Neurobehavioral responses of the freshwater teleost *Cyprinus carpio* (Linnaeus.) under quinalphos intoxication. Biotechnol. Anim. Hus. 25, 241-249.

Coats, J.R., O.Donnel-Jeffery, N.L., 1979. Toxicity of four synthetic pyrethroid insecticides to rainbow trout. Bull. Environ. Conton. Toxicol. 23, 250-255.

Colgan, M., Katch. F.I., 1986. The effects of micronutrient supplementation on athletic performance. Sport, Health and nutrition. Human Kinetics 1986, 21–50.

Cortés-Gutiérrez, E.I., López-Fernández, C., Fernández, J.L., Dávila-Rodríguez, M.I., Johnston, S.D., Gosálvez, J., 2014. Interpreting sperm DNA damage in a diverse range of mammalian sperm by means of the two-tailed comet assay. Fgene. 5(404), 1-11.

Cuesta, A., Esteban, M.A., Meseguer, J., 2002. Natural cytoxicity activity in seabream (*Sparus aurata* L.) and its modulation by vitamin C. Fish Shellfish Immunol. 13, 97-109.

Dabrowski, K. 1990. Ascorbic acid status in the early life of whitefish (*Coregonus lavaretus* L.). Aquaculture 84, 61–70.

Das, B. K., Mukherjee, S.C., 2003. Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatic and haematological consequences. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 134(1), 109–121.

Datta, M., Kaviraj, A., 2003. Ascorbic acid supplementation of diet for reduction of deltamethrin induced stress in freshwater catfish *Clarias gariepinus*. Chemosphere 53, 883–888.

David, M., Mushigeri, S.B., Shivakumar, R., Philip, G.H., 2005. Response of *Cyprinus carpio* (Linn) to sublethal concentration of cypermethrin: alterations in protein metabolic profiles. Chemoshpere 56, 347-352.

Denison, D.A., Koehn, R.D., 1977. Cellulase activity of *Poronia oedipus*. Mycologia 69(3), 592–603.

Deshpande, S.M., Aher., K.R., 2012. Evaluation of groundwater quality and its sustainability for drinking and agriculture use in parts of Vaijapur district Aurangabad, MS, India. Res. J. Chem. Sci. 2(1), 25-31.

Dizdaroglu, M., Nackerdien, Z., Chao, B.C., Gajewski, E., Rao, G., 1991. Chemical nature of in vivo DNA base damage in hydrogen peroxide-treated mammalian cells. Arch. Biochem. Biophys. 285(2),388–390.

Doyotte, A., Cossu, C., Jacquin, M.C., Babut, M., Vasseur, P., 1997. Antioxidant enzymes, glutathione and lipid peroxidation as relevant biomarkers of experimental or field exposure in the gills and the digestive gland of the freshwater bivalve *Unio tumidus*. Aquat. Toxicol. 39, 93–110.

Dunier, M., Vergnet, C., Siwicki, A.K., Verlhac, V., 1995. Effect of lindane exposure on rainbow trout (*Oncorhynchus mykiss*) immunity. IV. Prevention of nonspecific and specific immunosuppression by dietary vitamin C (ascorbate-2-polyphosphate). Ecotoxicol. Environ. Saf. 30, 259-268.

Durve, V.S., Lovell, R.T., 1982. Vitamin C and disease resistance in channel catfish (*Ictalurus punctatus*). Can. J. Fish. Aquat. Sci. 29, 948-951.

Edwards, R., Miliburn, P., Hustan, D.H., 1986. Comparative toxicity of cis-cypermethrin in rainbow trout, frog, mouse and quail. Toxicol. Appl. Pharmacol. 84, 512-522.

Edwards, R., Millburn, P., 1985. Toxicity and metabolism of cypermethrin in fish compared with other vertebrates. Pestic. Sci. 16, 201-202.

EL-Beltagi, H.S., Mohamed, H.I., 2013. Reactive oxygen species, lipid peroxidation and antioxidative defense mechanism. Not Bot Horti Agrobo. 41, 44-57.

Elia, A.C., Waller, W.T., Norton, S.J., 2002. Biochemical responses of Bluegill sunfish (*Lepomis macrochirus*, Rafinesque) to atrazine induced oxidative stress. Bull. Environ. Contam. Toxicol. 68, 809-816.

El-Sayed, Y.S., Saad, T.T., 2008. Subacute intoxication of a deltamethrin-based preparation (Butox 5% EC) in monosex Nile tilapia, *Oreochromis niloticus* L. Bas. Clin. Pharmacol. Toxicol. 102(3), 293–299.

Espinoza-Qui˜nones, F.R., Zacarkim, C.E., Palacio, S.M., Obreg´on, C.L., Zenatti, D.C., Galante, R.M., Rossi, N., Rossi, F.L., Pereira, I.R.A., Welter, R.A., 2005. Removal of heavy metal from polluted river water using aquatic macrophytes *Salvinia* sp. Braz. J. Phy. 35(3), 744–746.

Fahmy, A.M., Abdallah, E.F., 2001. Cytogenetic effects by the natural pyrethrins and the synthetic lambda-cyhalothrininmice in vivo. Cytologia 66, 139–149.

Farombi, E.O., Adelowo, O.A., Ajimoko, Y.R., 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African catfish (*Clarias gariepinus*) from Nigeria Ogun River. Int. J. Environ. Res. Pub. Health 4, 158-165.

Fazio, F., Piccione, G., Tribulato, K., Ferrantelli, V., Giangrosso, G., Arfuso, F., Faggio, C., 2014. Bioaccumulation of Heavy Metals in Blood and Tissue of Striped Mullet in Two Italian Lakes. J. Aquat. Anim. Health 26, 278–284.

Fetoui, H., Garoui, E.M., Makni-ayadi, F., Zeghal, N., 2008. Oxidative stress induced by lambda-cyhalothrin (LTC) in rat erythrocytes and brain: Attenuation by vitamin C. Environ. Toxicol. Pharmacol. 26, 225-231.

Fetoui, H., Garoui, E.M., Zeghal, N., 2009. Lambda-cyhalothrin-induced biochemical and histopathological changesin the liver of rats: Ameliorative effect of ascorbic acid. Experimen. Toxicol. Pathol. 61: 189-196.

Fetoui, H., Makni, M., Garoui, E.M., Zeghal, N., 2010. Toxic effects of lambda-cyhalothrin, a synthetic pyrethroids pesticide, on the rat kidney: Involvement of oxidative stress and protective role of ascorbic acid. Exp. Toxicol. Pathol. 62, 593-599.

Filho, D.W., Torres, M.A., Tribess, T.B., Pedresosa R.C., Soares, C.H.L., 2001. Influence of season and pollution on the antioxidant defenses of the cichlid fish acará (*Geophagus brasiliensis*). Braz. J. Med. Biol. Res. 34, 719-726.

Finney, D.J., 1971. Probit Analysis (3rd Edn.). Cambridge University Press, London.

FMC Corporation., 1989. Material safety data sheet Pounce (R) 1.5 G insecticide. Philadelphia, USA.

FoMonteiro, D.A., Almeida, J.A., Rantin, F.T., Kalinin, A.L., 2006. Oxidative stress biomarkers in the fresh water characid fish, *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (methylparathion). Comp. Biochem. Physio. C. Toxicol. Pharmacol. 143(2), 141-149.

Fournier, D., Bride, J.M., Poirie, M., Berge, J.B., Plapp. F.W., 1992. Insect glutathione S-transferases. Biochemical characteristics of the major forms of houseflies susceptible and resistant to insecticides. J. Biol. Chem. 267(3), 1840-1845.

Franciscato, C., Moraes-Silva, L., Duarte, F.A., Oliveira, C.S., Ineu, R.P., Flores, E.M.M., Dressler, V.L., Peixoto, N.C., Pereira, M.E., 2011. Delayed biochemical changes induced by mercury intoxication are prevented by zinc pre-exposure. Ecotoxicol. Environ. Saf. 74(3), 480-486.

Freitas, R.M., Nascimento, V.S., Vasconcelos, S.M.M., 2004. Catalase activity in cerebellum, hippocampus, frontal cortex and striatum after status epilepticus induced by pilocarpine in Wistar rats. Neurosci Lett. 365, 102–105.

Friberg-Jensen, U., Wendt-Rasch, L., Woin, P., Christoffersen, K., 2003. Effects of the pyrethroid insecticide, cypermethrin, on a freshwater community studied under field conditions. I. Direct and indirect effects on abundance measures of organisms at different trophic levels. Aquat. Toxicol. 63, 357-371.

Gagnaire, B., Thomas-Guyon, H., Renault, T., 2004. In vitro effects of cadmium and chromium on pacific oyster, *Crassostrea gigas* (Thunberg) haemocytes. Fish Shellfish Immunol. 10(4), 502-512.

Garcia, F., Pilarski, F., Onaka, M., de Moraes, R., Martins, L., 2007. Hematology of *Piaractus mesopotamicus* fed diets supplemented with vitamins C and E, challenged by *Aeromonas hydrophila*. Aquaculture 271, 39–46.

Ghorbani, R.F., Abbasi, F., Molaei, M., Naeimi, A., 2013. Identification and distribution of fish fauna in Kaboodval stream (Golestan provinces, Iran). World J. Fish Mar.Sci. 5(5), 467-473.

Giray, B., Gurbay, A., Hincal, F., 2001. Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin E and allopurinol. Toxicol. Lett. 118,139-146.

Gül, S., Belge-Kurutas, E., Yıldız, E.A., Doran, F., 2004. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyprinidae) living in Seyhan Dam Lake, Turkey. Environ. Int. 30, 605-609.

Gupta, P.K., 1989. Pesticide production in India: an overview. In: Mishra, P.C. (Ed), Soil Pollution and Soil Organisms. Ashish Publishing House, New Delhi, pp. 1–16.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139.

Halliwell, B., 1987. Oxidants and human disease: some new concepts. FASEB J. 1(5), 358-364.

Halliwell, B., Arouma, O. I., 1991. DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. FEBS Lett. 281, 9-19.

Halliwell, B., Gutteridge, J.M.C., 1989. Free radicals in biology and medicine. 2nd ed. Oxford, Clarendon Press.

Hardie, L.J., Fletcher, T.C., Secombes, C.J., 1991. The effect of dietary vitamin C on the immune responses of the Atlantic salmon (*Salmo salar* L.). Aquaculture 95, 201-214.

Hart, K., Pimentel, D., 2002. Public health and costs of pesticides. Encyclopedia of Pest Management, New York.

Hasspieler, B.M., Behar, J.V., Di Giulio, R.T., 1994. Glutathione dependent defense in channel catfish (*Ictalurus punctatus*) and brown bullhead (*Ameiurus nebulosus*). Ecotoxicol. Environ. Sal. 28(1), 82-90.

Hermes-Lima, M., 2004. Oxygen in biology and biochemistry: role of free radicals. In: Functional Metabolism: Regulation and Adaptation. Storey, K.B., (editor). John Wiley & Sons; New Jersey pp.319-368.

Hurst, J.K., Barrette, W.C., 1989. Leukocytic oxygen activation and microbicidal oxidative toxins. Crit. Rev. Biochem. Mol. Biol. 24: 271-328.

Iqbal, K., Khan, A., Khattak, M.M.A.K., 2004. Biological significance of ascorbic acid (vitamin C) in human health–a review. Pak. J. Nutr. 3, 5–13.

Jabeen, G., Javed, M., Azmat, H., 2011. Assessment of heavy metals in the fish collected from the River Ravi, Pakistan. Pak. Vet. J. 32(1), 107-111.

Jain, P., Sharma, J.D., Sohu, D., Sharma, P., 2005. Chemical analysis of drinking water of villages of Sanganer Tehsil, Jaipur district. Int. J. Environ. Sci. Technol. 2(4): 373-379.

Jakeman, P., Maxwell, S., 1993. Effects of antioxidant vitamin supplementation on muscle function after eccentric exercise. Eur. J. Appl. Physiol. 67, 426–430.

Jee, J.H., Masroor, F., Kang, J.C., 2005. Responses of cypermethrin-induced stress in haematological parameters of Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Aquacult. Res. 36(9), 898–905.

John, P.J., Prakash, A., 2003. Bioaccumulation of pesticides on some organs of freshwater catfish *Mystus vittatus*. Bull. Environ. Contam. Toxicol. 70, 1013–1016.

Johnson, W.W., Finley, M.T., 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. United states Department of the interior fish and wild life service/Resource Publication 137, Washington,DC.

Jokanovic, M., 2001. Biotransformation of organophosphorus compounds. Toxicol. 166(3), 139-160.

Jollow, D.J., Michell, J.R., Zampaglione, N., Gillete, J.R., 1974. Bromoibenzene-induced Liver necrosis: Protective role of glutathione and evidence for 3,4-Bromobenzene oxide as hepatotoxic metabolite. Pharmacol. 11, 151-169.

Kakkar, P., Das, B., Viswanathan , P.N., 1984. A modified spectrophotometric assay of superoxide dismutase. Ind. J. Biochem. 21, 130-132.

Kale, M., Rathore, N., John, S., Bhatnagar, D., 1999. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. Toxicol. Lett. 105, 197–205.

Kannan, M., Muthusamy, P., Venkatachalam, U., 2014. Response of synthetic pyrethroid cypermethrin (10% EC) induced stress in biochemical and haematological parameters of indian major carp *Catla catla* (Hamilton, 1822). World J. Pharma. Res. 3(4), 1976-1996.

Kaur, M., Atif, F., Ansari, R.A., Ahmad, F., Raisuddin, S., 2011. The interactive effect of elevated temperature on deltamethrin induced biochemical stress responses in *Channa punctata* Bloch. Chem Biol Interact. 193, 216-224.

Kaviraj, A., Gupta, A., 2014. Biomarkers of Type II synthetic pyrethroids pesticides in Freshwater Fish. Biomed Res. Int. 2014, 1-7.

Khan, B.A., Farid, A., Khan, N., 2006. Survey of pesticide use on fruits and vegetables in district Peshawar. Sarhad J. Agri. 22, 497-501.

Kopecka, J., Rybakowas, A., Pempkowiak, J., 2004. AChE levels in mussels and fish collected off Lithuania and Poland (southern Baltic). Ocenologia 46, 405-418.

Köprücü, S.S., Köprücü, K., Ural, M.S., Ispir, U., Pala, M., 2006. Acute toxicity of organophosphorous pesticide Diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (*Silurus glanis* L.). Pest. Biochem. Physiol. 86, 99-105.

Köprücü, S.S., Yonar, E., Seker, E., 2008. Effects of Deltamethrin on antioxidant status and oxidative stress biomarkers in freshwater mussel *Unio elongatulus euchres*. Bull. Environ. Contam. Toxicol. 81, 253-257.

Korkmaz, N., Cengiz, E.I., Unlu, E., Uysal, E., Yanar, M., 2009. Cypermethrin- induced histopathological and biochemical changes in Nile Tilapia (*Oreochromis niloticus*) and the protective and recuperative effect of ascorbic acid. Environ. Toxicol. Pharmacol. 28, 198-205.

Kosygin, L., Dhamendra, H., Gyaneshwari, R., 2007. Pollution status and conservation strategies of Moirang River, Manipur with a note on its aquatic bio-resources. J. Environ. Biol. 28(3), 669-673.

Kousar, S., Javed, M., 2015. Diagnosis of metals induced DNA damage in fish using comet assay. Pak. Vet. J. 35(2), 168-172.

Kumar A., Sharma B., Pandey R.S., 2012. Assessment of stress in effect to pyrethroid insecticides, λ-cyhalothrin and cypermethrin, in a freshwater fish, *Channa punctatus* (Bloch). Cel. Mol. Biol. 58(1), 153–159.

Kumar, A., Vibudh, P.K., Parimal, K.K., 2013. Fish micronucleus assay to assess genotoxic potential of arsenic at its guideline exposure in aquatic environment. BioMetals 26, 337-346.

Kumaragura, A.K., Beamish, F.W.H., 1981. Lethal toxicity of permethrin (NRDC–143) to rainbow trout *Salmo gairdneri* in relation to body weight and temperature. Water Res. 15, 503–505.

Laabs, V., Amelung, W., Pinto, A., Wantzen, M., da Silva, C., Zech, W., 2002. Pesticides in surface, sediment, and rainfall of the northeastern Pantanal Basin. Braz. J. Environ. Qual. 31, 1636–1648.

Langiano, V.C., Martinez, C.B., 2008. Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 147, 222-231.

Lee, R.F., Steinert, S., 2003. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. Mutat. Res. 544, 43–64.

Lee, Y.L., Hardy, H.A., 1965. Influence of thyroid hormone on L-glycerophosphate dehydrogenase and other dehydrogenases in various organs of rats. J. Biol. Chem. 240, 1427-1452.

Lesko, L.J., Brousseau, D., Canada, A. T., Eastwood, G., 1980. Temporal variations in trough serum theophylline concentrations at steady state. J. Pharm. Sci. 69, 358-359.

Li, Y., Lovell, R.T., 1985. Elevated level of dietary ascorbic acid increase immune response in channel catfish. J. Nutr. 115, 123-131.

Lin, M., Shi-Yen, S., 2005. Dietary L-ascorbic acid affects growth, nonspecific immune responses and disease resistance in juvenile grouper, *Epinephelus malabaricus*. Aquaculture 244, 215–221.

Liu, P.R., Plumb, J.A., Guerin, M., Lovell, R.T., 1989. Effect of megalevels of dietary vitamin C on the immune response of channel catfish *Ictalurus punctatus* in ponds. Dis. Aquat. Org. 7, 191-194.

Loteste, A., Scagnetti, J., Simoniello, M.F., Campana, M., Parma, M.J., 2013. Hepatic enzymes activity in the fish *Prochilodus lineatus* (Valenciennes, 1836) after sublethal cypermethrin exposure. Bull. Environ. Contam. Toxicol. 90(5), 601–604.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randal, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193(1), 265-275.

Mahaboob K., Sobti, R.C., Kataria, L., 2005. Pesticide-induced alteration in mice hepato-oxidative status and protective effects of black tea extract. Clinica Chimica Acta 358, 131–138.

Malik, G.M., Joshi, M.P., Zadafiya, S.K., Raval, V.H., 2012. Study on physicochemical characterization of some lotic system of south Gujarat, India. Res. J. Chem. Sci. 2(1), 83-85.

Manjunatha, B., Mohiddin, G.J., Ortiz, J., Selvanayagam., 2014. Effect of exposure to sublethal concentrations of sodium cyanide on the biochemical aspects in liver of the fresh water fish, *Labeo rohita*. Int. J. Pharmacol. Pharma. Sci. 2(1), 7-15.

Marigoudar, S.R., 2012. Cypermethrin induced pathophysiological and some biochemical changes in the freshwater Teleost, *Labeo rohita* (Hamilton). PhD thesis submitted to Department of Zoology, Karnatak University, Dharwad-580 003, India.

Marigoudar, S.R., Ahmed, R.N., David, M., 2009. Cypermethrin induced respiratory and behavioural responses in *Labeo rohita*. Vet. Arch. 79, 583-590.

Marigoudar, S.R., Ahmed, R.N., David, M., 2010. Cypermethrin induced: In vivo inhibition of the acetylcholinesterase activity in functionally different tissues of the freshwater teleost, *Labeo rohita* (Hamilton). Toxicol. Environ. Chem. 91(6), 1175-1182.

Marigoudar, S.R., Ahmed, R.N., David, M., 2012. Ultrastructural responses and oxidate stress induced by cypermethrin in liver of *Labeo rohita*. Chem. Ecol. iFirst, 1-13.

Masud, S., Singh, I.J., 2013. Effect of Cypermethrin on some haematological parameters and prediction of their recovery in a freshwater Teleost, *Cyprinus carpio*. African J. Biotechnol. 7(9), 852-856.

Medina, M., Barata, C., Telfer, T., Baird, D., 2002. Age-and sex related variation in sensitivity to the pyrethroid cypermethrin in the marine copepod *Acartia tonsa* Dana. Arch. Environ. Contam. Toxicol. 42(1), 17–22.

Medina, M., Barata, C., Telfer, T., Baird, D., 2003. Continuous versus pulsed exposures in the environmental risk assessment of pesticides in marine aquaculture. Arch. Environ. Contam. Toxicol. 58, 236-245.

Meier, W., Schmitt, M., Wahli, T., 1991. Efficacy of ascorbic acid and ascorbate-2-monophosphate on viral hemorrhagic septicemia in rainbow trout (*Oncorhynchus mykiss*). In: Proceedings of the European Association of Fish Pathology. European Association of Fish Pathology, Budapest. pp: 68-83.

Meitei, N.S., Bhargava, V., Patil, P.M., 2004. Water quality of Purna River in Purna town, Maharastra state. J. Aqua. Biol. 19, 77-78.

Mello Filho, A.C., Hoffman, E., Meneghini, R., 1984. Cell killing and DNA damage by hydrogen peroxide are mediated by intracellular iron. Biochem. J. 218, 273–275.

Michelangeli, F., Robson, M.J., East, J.M., Lee, A.G., 1990. The conformation of pyrethroids bound to lipid bilayers. Biochem. Biophys. Acta 1028, 49–57.

Misra, K., Das, K., Mukherjee, C., Pradhan, J., 2007. Effects of dietary vitamin C on immunity, growth and survival of Indian major carp *Labeo rohita* fingerlings. Aquacult. Nutr. 13, 35–44.

Mohandas, J., Marshall, J.J., Duggin, G.G., Horvath, J.S., Tiller, D., 1984. Differential distribution of glutathione and glutathione related enzymes in rabbit kidney: Possible implications in analgesic neuropathy. Cancer Res. 44, 5086–5091.

Mommsen, T.P., Walsh P.J., 1992. Biochemical and environmental perspectives on nitrogen metabolism in fishes. Experientia 48, 583–593.

Montanha, F.P., Fredianelli, A.C., Wagner, R., Sacco, S.R., Rocha, D.C.C., Pimpao, C.T., 2014. Clinical, biochemical and haemathological effects in *Rhamdia quelen* exposed to Cypermethrin. Arq. Bras. Med. Vet. Zootec. 66(3), 697-704.

Montero, D., Marrero, M., Izquierdo, M.S., Robaina, L., Vergara, J.M., Tort, L., 1999. Effect of vitamin E and C dietary supplementation on some immune parameters of gilthead seabream (*Sparus aurata*) juveniles subjected to crowding stress. Aquaculture 171, 269-278.

Murray, R.K., Granner, D.K., Mayes, P.A., Rodwell, V.W., 2007. In: Harper's illustrated biochemistry. 26th Edition. The McGraw–Hill.

Murthy, K.S., Kiran, B.R., Venkateshwarlu, M., 2013. A review on toxicity of pesticides in Fish. Int. J. Open Sci. Res. 1(1), 15-36.

Mustapha, M.K., 2008. Assessment of the Water Quality of Oyun Reservoir, Offa, Nigeria, Using Selected Physico-Chemical Parameters. Turkish J. Fish. Aquat. Sci. 8(2), 309-319.

Naeem, M., Salam, A., Tahir, S.S., Rauf, N., 2010. Assessment of the essential element and toxic heavy metals in hatchery reared *Oncorhynchus mykiss*. Int. J. Agric. Biol. 12(6), 935-938.

Nafees, M., Jan, M.R., 2009. Residues of cypermethrin and endosulfan in soils of Swat valley. Soil Environ. 28(2), 113-118.

Narra, M.R., Rajender, K., Reddy, R.R., Rao, J.V., Begum, G., 2015. The role of vitamin C as antioxidant in protection of biochemical and haematological stress induced by chlorpyrifos in freshwater fish *Clarias batrachus*. Chemosphere 132, 172-178.

Navarre, O., Halver, J.E., 1989. Disease resistance and humoral antibody production in rainbow trout fed high levels of Vitamin C. Aquaculture 79, 207-221.

Nayak, S.K., Swain, P., Mukherjee, S.C., 2007. Effect of dietary supplementation of probiotic and vitamin C on the immune response of Indian major carp, *Labeo rohita* (Ham.). Fish Shellfish Immunol. 23, 892-896.

Neeraja, S.R.K., Giridhar, P., 2014. Effect of deltamethrin on some aspects of protein metabolism in fresh water fish *Labeo rohita* (hamilton) Int. J. Pharm. Pharm. Sci., 6, 494-497.

Nelson, D.L., Cox M.M., 2005. Lehninger principles of biochemistry, 3rd edition. Macmillan worth Publishers. New York, USA.

Niki, E., Noguchi, N., Tsuchihashi, H., Gotoh, N., 1995. Interactions among vitamin C, vitamin E and b-carotene. Am. J. Clin. Nutr. 62, 1322–1326.

OECD., 1996. Capacity development in environment. Proceedings of the IIED/OECD workshop. pp.301.

Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13(2), 57-149.

Ortuno, J., Cuest, A., Esteban, M.A., Meseguer, J., 2001. Effect of oral administration of high vitamin C and E dosages on the gilthead seabream (*Sparus aurata* L.) innate immune system. Vet. Immunol. Immunopath. 79, 167-180.

Ortuno, J., Esteban, M.A., Meseguer, J. 1999., Effect of high dietary intake of vitamin C on non-specific immune response of gilthead seabream (*Sparus aurata* L.). Fish Shellfish Immunol. 9, 429-443.

Oudou, H.C., Alonso, R.M., Bruun, H.H.C., 2004. Voltammetric behavior of the synthetic pyrethroid lamda cyhalothrin and its determination in soil and well water. Anal. Chim. Acta 523, 69–74.

Pandey, S., Parvez, S., Sayeed, I., Haque, R., Bin-Hafeez, B., Raisuddin, S., 2003. Biomarkers of oxidative stress: a comparative study of river Yamuna fish *Wallago attu* (Bl. & Schn.). Sci. Total Environ. 309, 105-115.

Panush, R.S., Delafuente, J.C., 1985. Vitamins and immunocompetence. World Rev. Nutr. Diet 45, 97-123.

Parma, M.J., Loteste, A., Campana, M., Bacchetta, C., 2007. Changes of hematological parameters in *Prochilodus lineatus* (Pisces, Prochilodontidae) exposed to sublethal concentration of cypermethrin. J. Environ. Biol. 28(1), 147-149.

Parthasarathy, R., Joseph, J., 2011. Study on the changes in the levels of protein metabolism in λ cyhalothrin-induced hepatotoxicity in fresh water Tilapia (*Oreochromis mossambicus*). Adv. Appl. Sci. Res. 2, 57-62.

Pfeuffer, R., 1999. Pesticide Surface Water Quality Report: January 1999 Sampling Event. South Florida Water Management District, West Palm Beach, Florida.

Prasad, B.G., Narayana, T.S., 2004. Subsurface water quality of different sampling stations with some selected parameters at Machilipatnam town. Nature Environ. Pollut. Technol. 3(1), 47-50.

Prashanth, M.S., 2003. Cypermethrin induced physiological and biochemical and histopathological changes in freshwater fish, *Cyprinus carpio*. Ph.D thesis, Karnataka University, Dharwad. p. 288.

Prashanth, M.S., David, M., 2010. Impact of Cypermethrin on Na^{+}–K^{+}, Ca^{2+} and Mg^{2+} ATPases in Indian Major Carp, *Cirrhinus mrigala* (Hamilton). Bull. Environ. Contam. Toxicol. 84, 80–84.

Prashanth, M.S., Neelagund, S.E. 2008. Impact of cypermethrin on enzyme activities in the freshwater fish *Cirrhinus mrigala* (Hamilton). Capian J. Env. Sci. 6(2), 91-95.

Prutz, W.A., Butler, J., Land, E.J., 1990. Interaction of Copper (I) with Nucleic Acids. Int. J. Rad. Biol. 58, 215–23

Purves, D., George, J.A., David, F., William, C.H., Anthony- Samuel, L., James, O.M., Leonard, E.W., 2008. Neuroscience. 4th edition. Sinauer Associates, Massachusetts, USA.

Ramana, R.M.V., Ramamurthi, R., 1983. Effect of sub lethal concentrations of mercuric chloride on nitrogen metabolism in the freshwater field crab, *Oxiotelphusa senex senex* (Fabricicus). J. Agna. Biol. 1, 8-14.

Ray, A., Banerjee, B.D., 1998. Stress, free radicals and the immune response: Modulation by drug. Arch. Pharmacol. 358, 739.

Reddy, A.T.V., Yellamma, K., 1991. Perturbations in carbohydrate metabolism during cypermethrin toxicity in fish, *Tilapia mossambica*. Biochem. Int. 23(4), 633– 638.

Reddy, M., Bashamonideen, M.D., 1989. Toxicity of synthetic pyrethroid insecticides fenvalerate and cypermethrin to the fish, *Cyprinus carpio*. Enriron. Eco. 7(4), 1016-1018.

Reddy, M.P., Bashamohideen, M., 1995. Alterations in protein metabolism in selected tissues of fish, *Cyprinus carpio*, during sublethal concentration of cypermethrin. Environ. Monit. Assess. 36, 183-190.

Reddy, M.S., Naik S., Bashamohideen, M.D., 1995. Toxicity of cypermethrin and permethrin to fish, *Cyprinus corpio*.; Envion and Ecol. 13(1), 30-33.

Reinke, L.A., Moore, D.R., Rau, J.M., McCay, P.B., 1995. Inorganic phosphate promotes redox cycling of iron in liver microsomes: effects on free radical reactions. Arch Biochem Biophys. 316, 758-764.

Rice, P.J., Drewes, C.D., Klubertanz, T.M., Bradbury, S.P., Coats, J.R., 1997. Acute toxicity and behavioral effects of chlorpyrifos, permethrin, phenol, strychnine, and 2,4-dinitrophenol to 30-day-old Japanese medaka (*Oryzias latipes*). Environ. Toxicol. Chem. 16, 696-704.

Rosety, M., Rosety-Rodríguez, M., Ordonez, F.J., Rosety, I., 2005. Time course variations of antioxidant enzyme activities and histopathology of gilthead seabream gills exposed to malathion. Histol. Histopathol. 20, 1017-1020.

Rowley, D.A., Halliwell, B., 1983. DNA damage by superoxide-generating systems in realtion to the mechanism of action of the antitumor activity. Arch. Biochem. Biophys. 761, 86-93.

Roy, N.K., 2002. Chemistry of Pesticides. CBS Publishers and Distributors, New Delhi, India.

Sagripanti, J.L., Kraemer, K.H., 1989. Site-specific oxidative DNA damage at polyguanosines produced by copper plus hydrogen peroxide. J. Biol. Chem. 264, 1729-1734.

Saha, S., Kaviraj, A., 2009. Effects of cypermethrin on somebiochemical parameters and its amelioration through dietary supplementation of ascorbic acid in freshwater catfish *Heteropneustes fossilis*. Chemosphere 74(9), 1254–1259.

Saha, S., Kaviraj, A., 2012. Dietary ascorbic acid as a means to counter the stress of cypermethrin on the growth of freshwater catfish *Heteropneustes fossilis*. Toxicol. Indust. Health 2012, 1-6.

Sakaida, I., Kyle, M.E., Farber, J.L., 1990. Autophagic degradation of protein generates a pool of ferric iron required for the killing of cultured hepatocytes by an oxidative stress. Mol. Pharmacol. 37(3), 435–442.

Sandhu, H.S., Brar, R.S., 2000. Textbook of Veterinary Toxicology. 1st Ed., Kalyani Publ., New Dehli, India, pp: 225-235.

Sankar, P., Telang, A.G., Manimaran, A., 2012. Protective effect of curcumin on cypermethrin-induced oxidative stress in Wistar rats. Exp Toxicol Pathol. 64, 487-493.

Santos, I.M.S., da Rocha Tome, A., Saldanha, G.B., Ferreira, P.M.P., Militao, G.C.G., de Freitas, R.M. 2009. Oxidative stress in the hippocampus during experimental seizures can be ameliorated with the antioxidant ascorbic acid. Oxid. Med. Cell. Longev. 2(4), 214-221.

Sarkar, S.K., Bhattacharya, B.D., Bhattacharya, A., Chatterjee, M., Alam, A., Satpathy, K.K., Jonathan, M.P., 2008. Occurrence, distribution and possible sources of organochlorine pesticide residues in tropical coastal environment of India: An overview. Environ. Int. 34(7), 1062-1071.

Sayeed, I., Parvez, S., Pandey, Sl., Bin-Hafeez, B., Haque, R., Raisuddin, S., 2003. Oxidative stress biomarkers of exposure to Deltamethrin in freshwater fish, *Channa punctatus* Bloch. Ecotoxicol. Environ. Saf. 56(2): 295-301.

Scalon, M.C.S., Rechenmacher, C., Siebel, A.M., Kayser, M.L., Rodrigues, M.T., Maluf, S.W., Rodrigues, M.A.S., Silva, L.B., 2010. Evaluation of Sinos River water genotoxicity using the comet assay in fish. Braz. J. Biol. 70, 1217-1222.

Schraufstätter, I., Hyslop, P.A., Jackson, J.H., Cochrane, C.G., 1988. Oxidant-induced DNA damage of target cells. J. Clin. Invest. 82(3), 1040-1050.

Shanbehzadeh, S., Dastjerdi, M.V., Hassanzadeh, A., Kiyanizadeh, T., 2014. Heavy metals in water and sediments: A case study of Tembi river. J. Environ. Pub. Health 014, 1-5.

Sharbidre, A.A., Vimal, M., Priyanka, P., 2011. Effect of Diazinon on acetylcholinesterase activity and lipid peroxidation of *Poecilia reticulata*. Res. J. Environ. Toxicol. 5, 152-161.

Shi, X., Gu, A., Ji, G., 2011. Developmental toxicity of Cypermethrin in embryo-larval stages of zebrafish. Chemosphere 85(6), 1010–1016.

Shobha, R.A.R., Sudharsan, T.N., Reddy, P.V.M., Reddy, R.T.N., 2001. Effect of arsenite on certain aspects of protein metabolism in freshwater teleost, *Tilapia mosambica* (Peters). J. Environ. Biol. 22, 101-104.

Sies, H., 1986. Biochemistry of oxidative stress. Angewandte Chemie- Int. Ed. Eng. 25, 1058-1071.

Sies, H., 1988. Oxidative stress: quinone redox cycling. ISI Atlas Sci. Biochem. 1, 109-114.

Sies, H., 1999. Glutathione and its role in cellular function. Free Radic. Biol. Med. 27(9-10), 916-921.

Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for the quantitation of low levels of DNA damage in individual cells, Exp. Cell Res. 175, 184-191.

Singh, S.K., Singh, S.K., Yadav, R.P., 2010. Toxicological and Biochemical Alterations of Cypermethrin (Synthetic Pyrethroids) Against Freshwater Teleost Fish *Colisa fasciatus* at Different Season. World J. Zool. 5(1), 25-32.

Sivaperumal, P., 2008. The influence of organophosphorus pesticide methylparathion On protein, lipid metabolism and Detoxifying enzymes in rohu (*Labeo rohita*). PhD thesis submitted to Cochin University Of Science And Technology Cochin-682 016, India.

Sivaperumal, P., Sankar, T.V., 2011. Toxic effects of methyl parathion on antioxidant enzymes and acetylcholinesterase activity in freshwater fish, *Labeo rohita*. Fishery Technol. 48, 59-66.

Smet, H.D., Blust, R., 2001. Stress Responses and Changes in Protein Metabolism in Carp *Cyprinus carpio* during Cadmium Exposure. Ecotoxicol. Environ. Saf. 48, 255-262.

Sohn, H.Y., Kwon, C.S., Kwon, G.S., Lee, J.B., Kim, E., 2004. Induction of oxidative stress by endosulfan and protective effect of lipid soluble antioxidants against endosulfan-induced oxidative damage. Toxicol. Lett. 151(2), 357–65.

Sram, R.J., Binkova, B., Rossner P.Jr., 2012. Vitamin C for DNA damage prevention. Mut. Res. 733, 39-49.

Sridevi, G., 1991. In vivo changes in the carbohydrate metabolism of freshwater fish, *Labeo rohita* under cypermethrin stress. M.Phil. dissertation, S.K. University, Anantapur, Andhra Pradesh, India.

Sthanadar, I.A., Begum, B., Sthanadar, A.A., Nasar, M.J., Ahmad, I., Zahid, M., Muhammad, A., Ullah, S., 2015. Bioaccumulation of heavy metals in intestine of Mulley (*Wallago attu*, Bloch & Schneider, 1801): A case study of Kalpani River at District Nowshera Khyber Pakhtunkhwa Pakistan. J. Biodiv. Environ. Sci. 6(2), 74-80.

Sulak, O., Altuntas, I., Karahan, N., Yildirim, B., Akturk, O., Yilmaz, H.R., 2005. Nephrotoxicity in rats induced by organophosphate insecticide emethidathion and ameliorating effects of vitamin C. Pestic. Biochem. Phys. 83, 21–28.

Tananchai, C., Tisgratog, R., Grieco, J.P., Chareonviriyaphap, T., 2012. Pyrethroid induced behavioral responses of *Anopheles dirus*, a vector of malaria in Thailand. J. Vector Ecol. 37(1), 187-196.

Tantarpale, S.A., 2011. Cypermethrin impact on total protein in muscle and liver of the freshwater fish *Channa striatus*. Sci. Res. Rep. 1, 155.

Tchounwou, P.B., Wilson, B., Ishaque, A., Ransome, R., Huang, M. J., Leszczynski, J., 2000. Toxicity assessment of atrazine and related triazine compounds in the microtox assay, and computational modeling for their structure-activity relationship. Int. J. Mol. Sci. 1, 63-74.

Thompson, I., White, A., Fletcher, T.C., Houlihan, D.F., Secombe, C.J., 1993. The effect of stress on the immune response of Atlantic salmon (*Salmo salar* L.) fed diets containing different amounts of vitamin C. Aquaculture 114, 1-18.

Tiwari, S., Tiwari, R., Singh, A., 2012. Impact of Cypermethrin on Fingerlings of Common Edible Carp (*Labeo rohita*). Scientific World J. 2012, 1-7.

Toyoizumi, T., Deguchi, Y., Masuda, S., Kinae, N., 2008. Genotoxicity and estrogenic activity of 3,3′ -Dinitrobisphenol A in Goldfish. Biosci. Biotechnol. Biochem. 72(8), 2118-2123.

Traber, M.G., Stevens, J.F., 2011. Vitamins C and E: Beneficial effects from a mechanistic perspective. Free Radical Biol. Med. 51, 1000-1013.

Tripathi, P.K., Singh, A., 2003. Toxic effects of dimethoate and carbaryl pesticides on protein metabolism of the freshwater snail *Lymnaea acuminata*. Bull. Environ. Contam. Toxicol. 70, 146–152.

UK Pesticides Action Network (PAN)., 2009. List of Lists: A Catalogue of Lists of Pesticides Identifying Those Associated with Particularly Harmful Health or Environmental Impacts. P. A. Network.

Ullah, R., Zuberi, A., Naeem, M., Ullah, S., 2014f. Acute Toxic Effects of Cypermethrin on Hematology and Morphology of Liver, Brain and Gills of Mahseer (*Tor putitora*). Int. J. Agri. Biol. 17(1), 199-204.

Ullah, R., Zuberi, A., Ullah, S., Ullah, I., Dawar, F.U., 2014e. Cypermethrin induced behavioral and biochemical changes in mahseer, *Tor putitora*. J. Toxicol. Sci. 39(6), 829-836.

Ullah, S., 2014. GIS Integrated approach for assessing drinking water quality. VDM Publishing, Heinrich-Böcking-Street, 6-8 D – 66121, Saarbrücken Germany. pp: 1-2.

Ullah, S., Javed, M.W., Muhammad, I., Ullah, W., 2014c. Physico-chemical analysis of tube wells' water at district Dir Lower, Khyber Pakhtunkhwa Pakistan. Pakhtunkhwa J. Life Sci. 2(1), 28-37.

Ullah, S., Javed, M.W., Shafique, M., Khan, S.F., 2014a. An integrated approach for quality assessment of drinking water using GIS: A case study of Lower Dir. J. Himalayan Earth Sci. 47(2), 163-174.

Ullah, S., Ullah, N., Ahmad, T., 2014d. Assessment of dug wells' water for drinking and irrigation purposes at district Dir Lower, Khyber Pakhtunkhwa Pakistan. Am. J. Life Sci. Res. 2(4), 466-473.

Ullah, S., Ullah, N., Rahman, K., Khan, T.M., Jadoon, M.A., Ahmad, T., 2014b. Study on physicochemical characterization of Konhaye stream District Dir Lower, Khyber Pakhtunkhwa Pakistan. World J. Fish Marine Sci. 6(5), 461-470.

Ullah, S., Zorriehzahra, M.J., 2014. Ecotoxicology: A review of pesticides induced toxicity in Fish. Adv. Anim. Vet. Sci. 3(1), 40-57.

UN., 2003. Water for People, Water for Life—UN World Water Development Report (WWDR), UNESCO and Berghahn Books, UN.

Üner, N., Oruç, E. O., Canli, M., Sevgiler, Y., 2001. Effects of cypermethrin on antioxidant enzyme activities and lipid peroxidation in liver and kidney of the freshwater fish, Oreochromis niloticus and Cyprinus carpio (L.). Bull Environ Contam Toxicol. 67, 657-664.

USCB., 2015. United States Census Bureau Report for Pakistan and Bangladesh, Available at:http://www.google.com.pk/publicdata/explore?ds=d5bncppjof8f9_&met_y=sp_pop_totl&idim=country:PAK:BGD&hl=en&dl=en (Accessed on June 30th, 2015).

USEPA., 2000. Reregistration eligibility science chapter for chlorpyrifos. Fate & environmental risk assessment chapter (Revised June). Accessed at July 5th, 2015. Available at: http://www.epa.gov/pesticides/op/chlorpyrifos/efedrra1.pdf.

Vani, T., Saharan, N., Mukherjee, C., Ritesh, R., Rajesh, K., Brahmchari, K., 2011. Deltamethrin induced alterations of hematological and biochemical parameters in fingerlings of *Catla catla* (Ham.) and their amelioration by dietary supplement of vitamin C. Pestic. Biochem. Physiol. 101, 16–20.

Vani, T., Saharan, N., Roy, S.D., 2012. Alteration in haematological and biochemical parameters of *Catla catla* exposed to sub-lethal concentration of Cypermethrin. Fish Physiol. Biochem. 38(6), 1577–1584.

Veeraiah, K., Durga-Prasad, M.K., 1998. Study on the toxic effects of Cypermethrin (technical) on organic constituents of freshwater fish, *Labeo rohita* (Hamilton). Proc. Acad. Environ. Biol. 7(2), 143-148.

Velisek, J., Stara, A., Kolarova, J., Svobodova, Z. 2012. Effects of long-term exposure to simazine in real concentrations on common carp (*Cyprinus carpio* L.). Ecotoxicol. Environ. Saf. 76, 79-86.

Velisek, J., Stara, A., Kolarova, J., Svobodova, Z., 2011. Biochemical, physiological and morfological responses in common carp (*Cyprinus carpio* L.) after long-term exposure to terbutryn in real environmental concentration. Pest. Biochem. Physiol. 100, 305-313.

Velisek, J., Wlasow, T., Gomulka, P., 2006. Effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*). Veterinarni Medicina 51(10), 469–476.

Verlhac, V., Obach, A., Gabaudan, J., Schuep, W., Hole, R., 1998. Immunomodulation by dietary vitamin C and glucan in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 8, 409-424.

Vidyunmala, S., Begum, A.F., Nagalakshmamma, K., Rao, A.V.B., 2010. Toxicity evaluation of Fluoride in Silk *Bombyx mori* L. Am-Eur. J. Toxicol. Sci. 2(2), 96-99.

Villacorta, L., Azzi, A., Zingg, J., 2007. Regulatory role of vitamins E and C on extracellular matrix components of the vascular system. Mol. Asp. Med. 28, 507-537.

Wahli, T., Meier, W., Pfister, K., 1986. Ascorbic acid induced immune-mediated decrease in mortality in *Ichthyophthirius multifiliis* infected rainbow trout (*Salmo gairdneri*). Acta Trop. 43, 287-289.

Walz, R., Moreira, J.C.F., Benfato, M.S., Quevedo, J., Schorer, N., Vianna, M.M.R., 2000. Lipid peroxidation in hippocampus early and late after status epilepticus induced by pilocarpine of kainic acid in Wistar rats. Neurosci. Lett. 291, 179–182.

Wast, N., Gupta, A.K., Prakash, M.M. and Gaherwal, S., 2014. Acute Toxicity of Chlorpyriphos 50% + Cypermethrin 5% EC to the Guppy, *Poecilia reticulata* (Peters, 1859). Global Veterinaria 12(3), 393-398.

WHO., 1989. Cypermethrin Environmental Health Criteria 82. International Programme on Chemical Safety, World Health Organization, Geneva Switzerland.

WHO., 1992. Alpha–cypermethrin. Environmental Health Criteria. World Health Organization, Geneva.

Wood, S., Sebastian, K., Scherr, S.J., 2000. Pilot analysis of global ecosystems: Agroecosystems. International Food Policy Research Institute and World Resources Institute, Washington D.C. USA.

Wright, J. R., Colby, H. D., Miles, P. R., 1981. Cytosolic factors that affect acrosomal lipid peroxidation in lung and liver. Arch. Biochem. Biophys. 206, 96-304.

Yavuz, T., Delibas, N., Yildirim, B., Altuntas, I., Candır, O., Cora, A.N., 2004. Vascular wall damage in rats induced by methidathion and ameliorating effect of vitamins E and C. Arch. Toxicol. 78, 655–659.

Yousaf, S., Zada, A., Owais, M., 2013. Physico-chemical characteristics of potable water of different sources in District Nowshera: A case study after flood – 2010. J. Himalayan Earth Sci. 46(1), 83-87.

Zhang, J., Shen, H., Wang, X., Wu, J., Xue, Y., 2004. Effects of chronic exposure of 2,4-dichlorphenol on the antioxidant system in liver of freshwater fish *Carassius auratus*. Chemosphere 55, 167-174.

Zhou, Q., Wang, L., Wang, H., Xie, F., Wang, T., 2012. Effect of dietary vitamin C on the growth performance and innate immunity of juvenile cobia (*Rachycentron canadum*). Fish Shellfish Immunol. 32, 969–975.