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Research Article

EFFECTS OF AGRO-CHEMICALS ON ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION IN *OREOCHROMIS MOSSAMBICUS* AND *LABEO ROHITA*

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ABSTRACT

In the present work, an attempt has been made to investigate the mechanisms of agrochemicals (Imidacloprid-IMI and Curzate-CZ) induced oxidative stress (OS) in key organs of agrochemicals exposed two teleost fishes. Major complications arise when the stressor is very effective and the body starts expressing mechanisms of stress response. They include lipid peroxidation (LPO) and expression of various antioxidant machineries like Glutathione S transferase (GST), Catalase (CAT), Superoxide dismutase (SOD) and Glutathione Peroxidase (GPx) as well as scavengers such as reduced Glutathione (GSH) and ascorbic acid (AA). Agrochemical stress significantly increased AA and GSH content in liver, kidney and gills in a dose dependent manner while, GPx level was found to be increased in gills and liver only. Organ-based GST assay reflected a dose dependent significant increase in liver, kidney and gills. Thus, from the present study it can be concluded that the response of antioxidant enzymes (SOD, CAT, GPx, and GST) and non-enzymatic antioxidant/scavengers (AA and GSH) showed that the both the teleost are under severe OS and that the agro-chemicals are acting as potent free radicals generators. LPO level proves that extensive lipid peroxidation has occurred on exposure of the agro-chemicals. And that both the antioxidants interact in a concerted manner to eliminate ROS and prevent damage to cellular components. This suggests that IMI and CZ at levels below median lethal concentration are capable of causing oxidative damage in *Oreochromis mossambicus* and *Labeo rohita*.

Keywords: *IMI, CZ, Oxidative stress, Reactive Oxygen species, Agrochemicals.*

INTRODUCTION

Agrochemicals in the form of insecticides, herbicides and fungicides are used extensively throughout the world. They are playing a pivotal role in meeting the food, cotton fibre and tobacco demand of escalating population and control of vector-borne diseases. Although they furnish some benefits for crop, they entail a number of risks and problems. Pesticide misuse in various sectors of the agriculture often has been associated with health problems and environmental contamination worldwide (Remor *et al.*, 2009). Misuse of highly toxic pesticides, coupled with a weak or a totally absent legislative framework in the use of pesticides, is one of the major reasons for the high incidence of pesticide

Developing countries use only 20% of the world's agrochemicals, yet they suffer 99% of deaths from pesticide poisoning (Atreya, 2008). The unregulated release of agricultural chemicals especially pesticides into water bodies have caused environmental problems to all classes of organisms in the aquatic habitat. The aquatic ecosystem is under threat of biodiversity loss due to indiscriminate use of pesticides. The application of environmental toxicology studies on non mammalian vertebrates is rapidly expanding, and for aquatic system, fish have become an indication for the evaluation of the effects of noxious compounds (Mensah *et al.*, 2014).Pesticides at high concentrations are known to reduce the survival, growth, and reproduction of fish and produce many visible effects on fish (Joseph and Raj, 2010).

Pesticide exposure can lead to oxidative stress (OS) through unregulated generation of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radical, peroxyl radicals and singlet oxygen. ROS are produced during normal process in the cell. Under

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normal conditions antioxidant systems of the cell minimize damage caused by ROS. When ROS generation increases to an extent that it overcomes the cellular antioxidant systems, the result is oxidative stress. Major complications arise when the stressor is very effective and the body starts expressing stress response, that include lipid peroxidation (LPO) and expression of various antioxidant mechanisms like GST, CAT, SOD and GPx and scavengers such as GSH and AA.

Several studies demonstrated that changes in the levels of antioxidant enzyme activities can be used as possible biomarkers in different aquatic organisms (Slaninova *et al*., 2014; Sepperumal and Saminathan, 2014). These enzymes are biomarkers of tissue damage, thus their bioassay can serve as a diagnostic tool for assessing the functions of vital organs. Hence, in the present work, an endeavor has been made to explore the pesticides induced OS, in various key organs of pesticide exposed fishes on lipid peroxidation as well as their antioxidant defense mechanisms as this aspect of the toxicity data for this new group of insecticides for aquatic invertebrate are far from enough.

MATERIALS AND METHODS

Experimental designs

Freshwater teleosts, *O. mossambicus* and *L. rohita* of similar size in length and weight (12 ± 2 cm; 25 ± 1.9 g) and (25 ± 3 cm; 110 ± 5 g) respectively were brought from a local pond of Baroda district. Animals were transported to laboratory in large aerated plastic container and were acclimatized in glass aquaria containing 50 liter of well aerated dechlorinated tap water (with physic-chemical characteristics: pH 6.5- 7.5, temperature 25±3ºC and dissolved oxygen content of 7-8 ppm) for ten days. During an acclimatization period of 10 days, the fish were kept under natural photoperiod (10:00 and 16:00h) and fed two times a day with commercial pellet diet. The acclimatized healthy fishes of both sexes were selected randomly for the studies Based on the result of the 48 h LC_{50}, 30 tilapia fish were divided in 3 groups, 10 fish for each group:

• Group 1 served as control without any treatment of Agro-chemicals.

• Group 2 were treated with low dose of IMI and CZ (LC_{50} / 10).

• Group 3 were treated with high dose of IMI and CZ (LC_{50} / 20) for a period of 21 days.

Each concentration was replicated two times. Constant amount of the test chemical and test media were changed every 24 hours to maintain the toxicant strength and the level of dissolved oxygen as well as to minimize the level of ammonia during experiment. The fishes were fed once in a day throughout the duration of the sub-lethal toxicity tests.

Preparation of the tissue samples for the study

At the end of the experiment (21 days) the fish were carefully netted to minimize stress, and weighed. Tissues such as liver, kidney, gills and muscle were carefully removed, wiped thoroughly, washed in chilled PBS After noting the total weight of the tissues, the desired amount of the tissues were weighed and used for quantitative analysis of the enzymes.

Estimation of AA was done following the method of Roe and Oesterling (1944); GSH was estimated by the method of Ellman and Fiches (1958); SOD was determined using the method of Kakkar *et al.*, (1984); CAT level was determined using the method of Maehly and Chance (1955); GPx was estimated by the method of Rotruck *et al.* (1973); GST was determined using the method of Beutler *et al.* (1986) and LPO was estimated by the method of Niehaus and Samuelson (1958).

Statistical Analysis

The statistical analysis was carried out using the software Graph pad prism 5 packages. For determining the significant difference between different treatments in biochemical parameters, Two-way ANOVA followed by Tukey's test for multiple comparisons between different concentration of IMI and CZ was done. Significance level (P value) was set at 0.05 in all tests.

RESULT

The effects of IMI and CZ exposure on various parameters of OS at high and low dose in *O. mossambicus* and *L. rohita* are shown in Figure 1 to 4. All the tissues, gills, liver, kidney and muscle showed significantly ($P<0.05$) elevated AA activity compared to control (Figure 1 A-D). There was significant $P<0.05$) variation in GSH content between treated groups and within tissues on exposure of IMI and CZ (Figure 1 E-H). Among the tissues, gills, liver and muscle illustrated significant ($P<0.05$) elevated activity compared to control but the kidney in both the treated groups showed significant ($P<0.05$) reduced activity compared to control. A dose dependent increase in the activity of SOD and CAT was found to in gills, liver and kidney of *O. mossambicus* and *L. rohita* on exposure of CZ and IMI (Figure 2 A-H) An increased GST activity ($P<0.05$) was seen in liver, but kidney and muscle expressed a significant ($P<0.05$) decrease in the GST (Figure 3 A-D). GPx activity too revealed an overall significant change ($P<0.05$), there was an increased activity in liver, kidney and muscles of the treated groups compared to control. Whereas gills, on exposure to IMI and CZ showed a decreased GPx activity compared to control (Figure 3 E-H). A significant elevation in LPO was noted in *L. rohita* and *O. mossambicus* on IMI exposure, however a non-significant increase was observed in *O. mossambicus* on exposure of CZ (Figure 4 A-D).

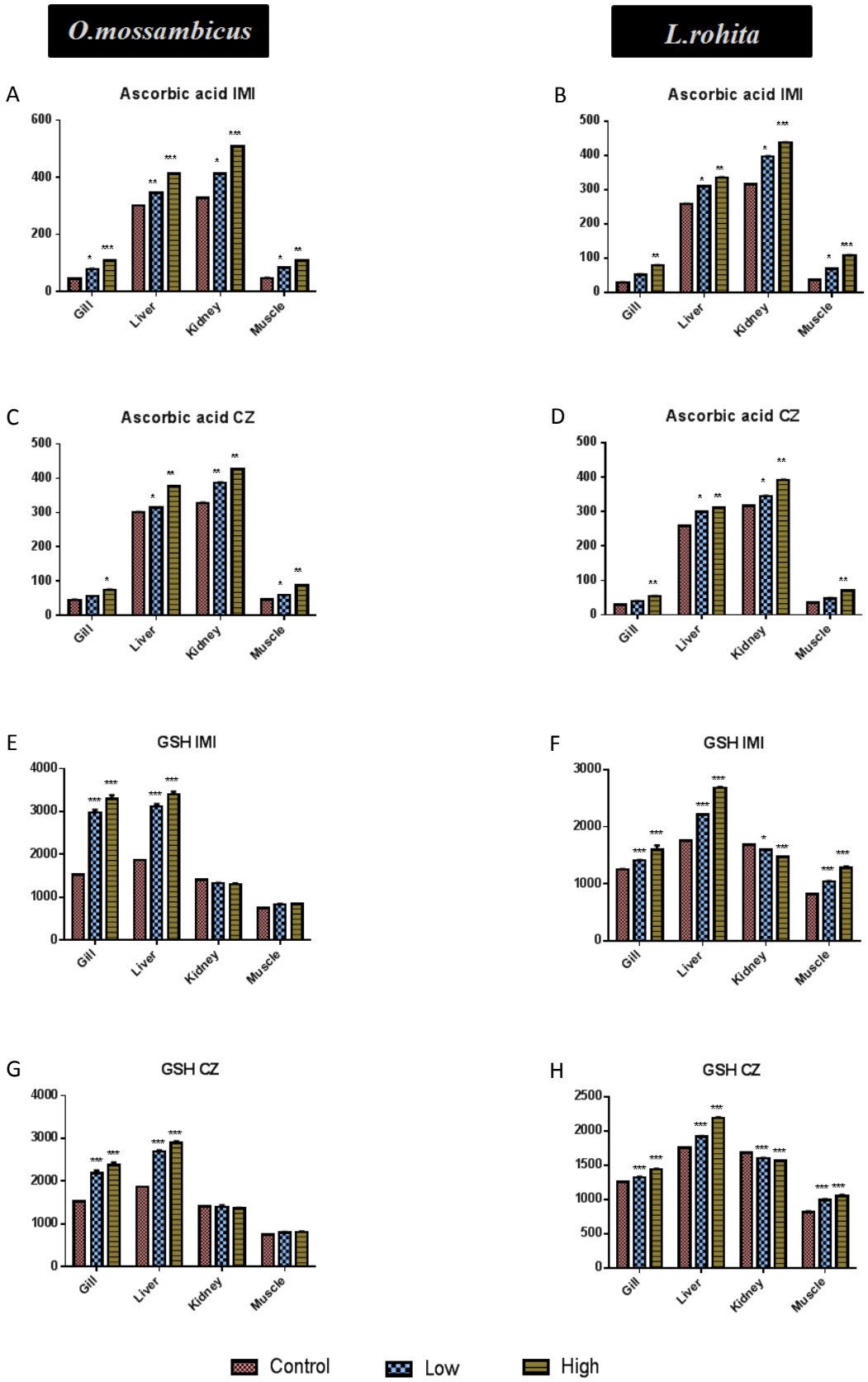

Figure 1. Effect of IMI and CZ on AA and GSH activity (mean ± SEM) in *O.mossambicus* (A, C, E, G) and *L. rohita* (B, D, F, H).

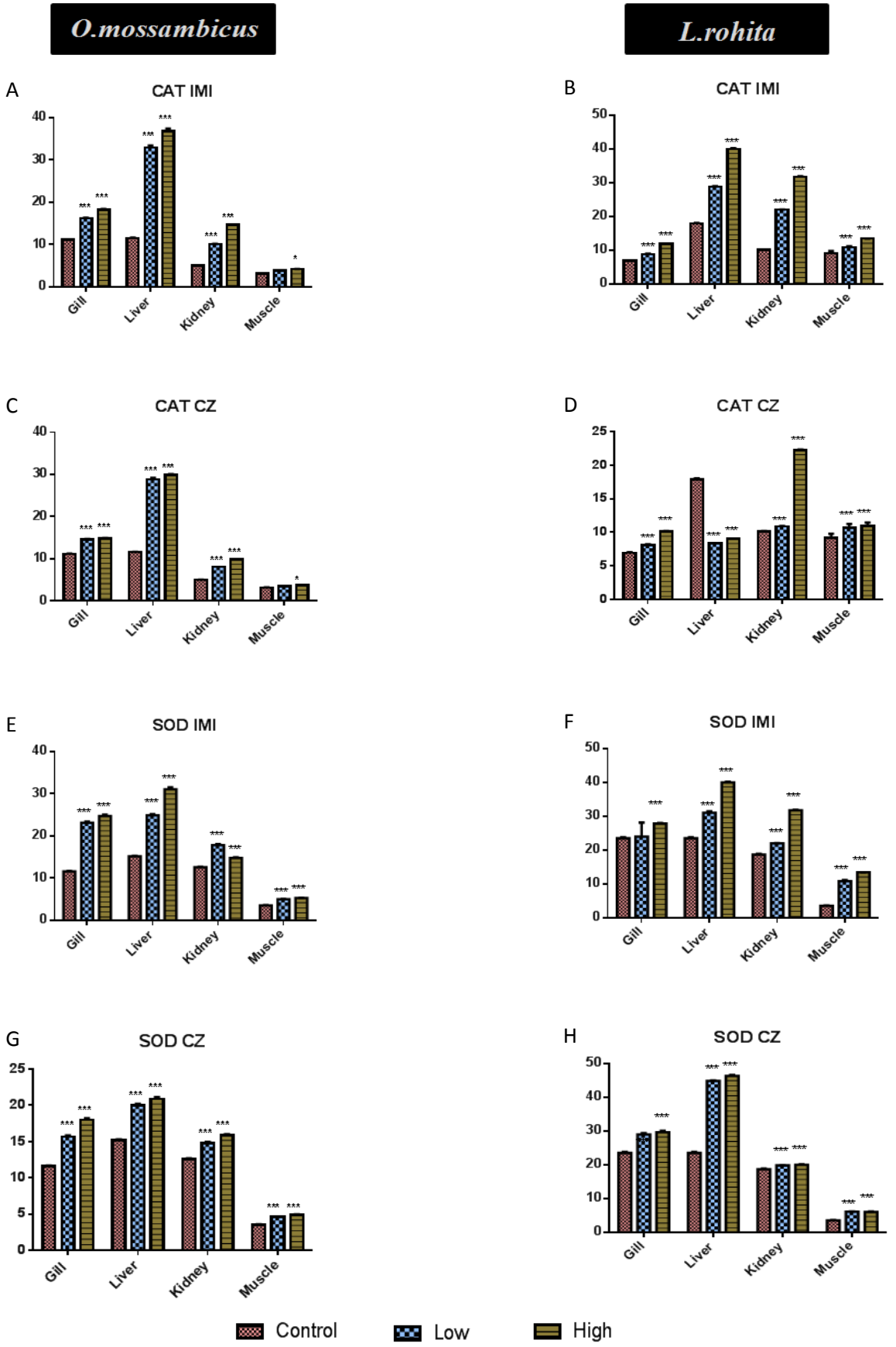

Figure: 2. Effect of IMI and CZ on CAT and SOD activity (mean ± SEM) in *O.mossambicus* (A,C,E,G) *and L.rohita* (B, D, F, H).

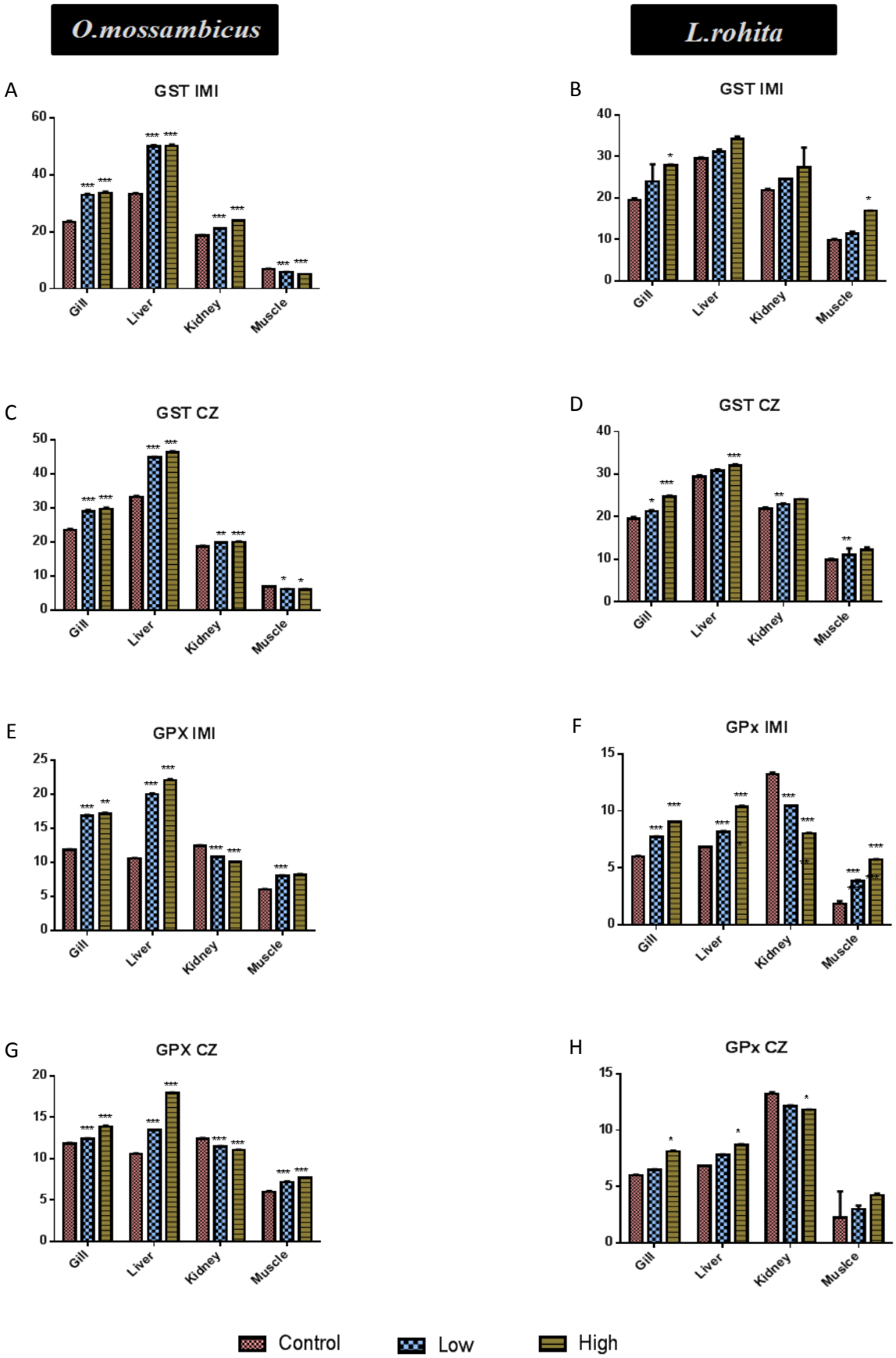

Figure 3. Effect of IMI and CZ on GST and GPx activity (mean ± SEM) *in O.mossambicus* (A, C, E, G) *and L.rohita* (B, D, F, H).

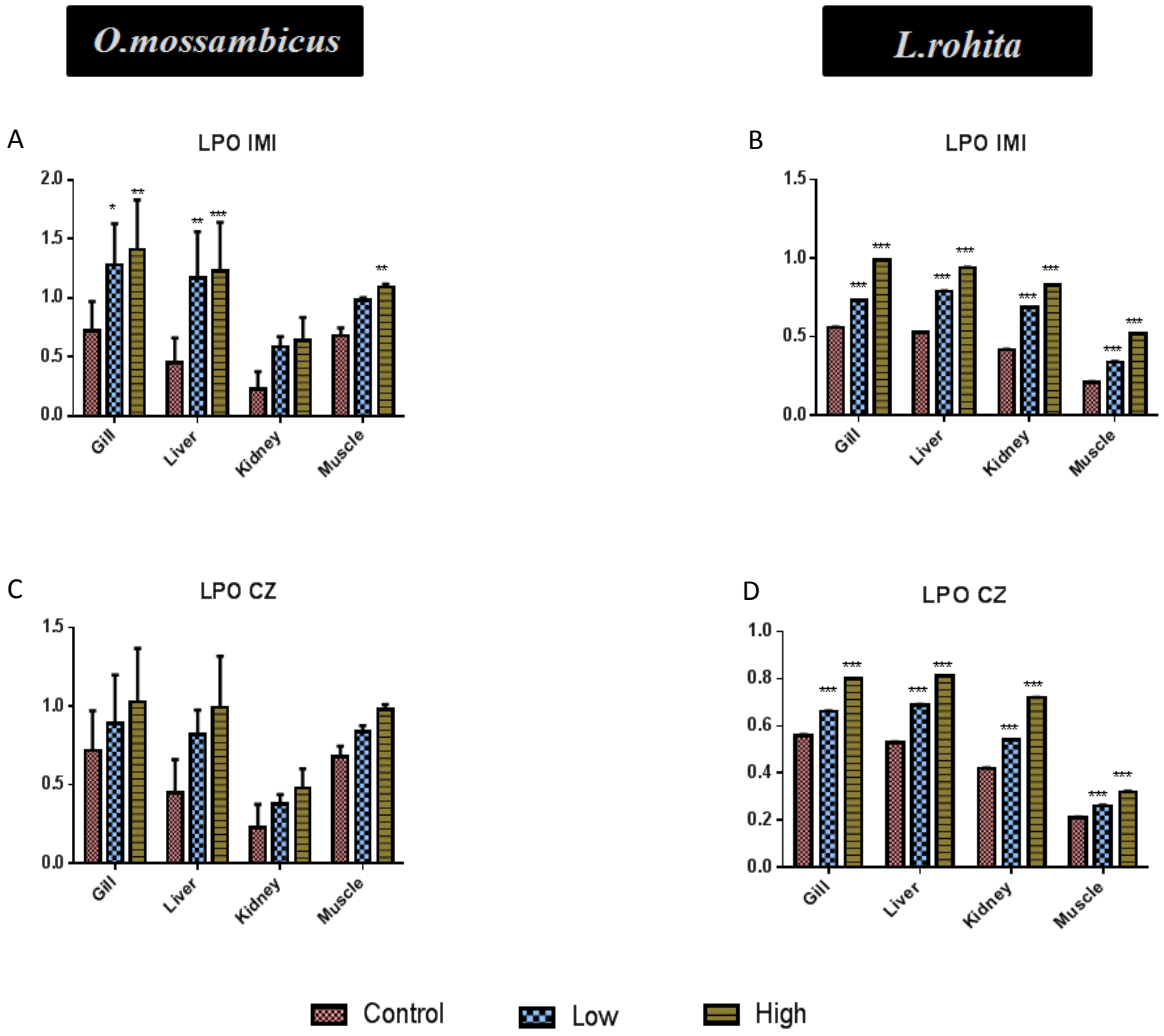

Figure: 4. Effect of IMI and CZ on LPO activity (mean ± SEM) of *O. mossambicus* (AS, C) and *L. rohita* (B, D).

DISCUSSION

Detoxification path at tissue level can be detected by biochemical markers of OS (Van der Oost *et al*., 2003). The first line of defence to oxidative stress is the use of antioxidant scavengers, such as AA (vitamin C), vitamin E, uric acid, carotenoid and GSH. In the present study agrochemical exposure has significantly increased AA content in liver, kidney and gills. AA protects host cells against harmful oxidants released into the extracellular medium. It can act as a hydrogen carrier as it has an essential role in the metabolism of protein; fats and carbohydrates. Due to its anti-oxidant role and as a part of redox buffer system increased AA is probably inhibiting the oxidative metabolism and preventing the production of electrophilic metabolites and is able to scavenge harmful free radical metabolites/ ROS. Hence, the high level of AA observed in the present study by agro-chemical induced stress condition is reasonable. The indication to detoxifying enzymes is reported to be accompanied by increase in AA content of liver, kidney and gills, which stimulate detoxification of toxicant, suggestive of liver, kidney and gills to be the sites of detoxification. Our results are in agreement with earlier reported elevated AA content in *O. mossambicus* (Guha and Khuda-Bukhsh, 2002); in *Clarias batrachus* (Kaviraj and Gupta, 2014) and *Puntius ticto* (Ganeshwade, 2011). Glutathione is a tri peptide that is mainly present in cells in its reduced form (GSH), which basically acts as an intracellular reductant and neucleophile (Vardharajan, 2010). It functions in the synthesis of proteins and DNA, free radical scavenging, as an essential cofactor of several enzymes, and as a defence against oxidizing molecules and potentially harmful pesticides (Dorval and Hontela, 2003). In the present study there was a significant increase in the GSH activity in liver and kidney on exposure of agrochemicals in a dose dependent manner. Among the tissues GSH level was found to be highest in the liver compared to other tissues which may be due to an adaptive mechanism to oxidative stress in its synthesis which can be provided for the increased GSH activity. However, a depletion of GSH was observed in kidney and muscles at low dose of IMI and CZ which illustrates that severe oxidative stress may have suppressed GSH levels due to loss of adaptive mechanisms leading to the oxidation of GSH to GSSG.

The second cellular mechanism to remove excess ROS and avoid oxidative damage is maintained through enzymatic defence strategy. These enzymatic defences include glutathione peroxidase (GPx), glutathione-s-transferase (GST), catalase (CAT) and superoxide dismutase (SOD). GPx is an enzyme with peroxidase activity and broad substrate spectrum (Lushchak, 2012). This enzyme is known to protect the fish from the damage caused by H_2O_2 and reduces it to lipid hydroperoxides (Mieiro *et al.,* 2011 Banaee, 2013). An increase in GPx activity in liver, kidney and gills is probably eliminating the access of H_2O_2 and lipid hydrogen peroxide produced in the fish exposed to agro-chemicals. Similar results have been observed in the liver of *Cyprinus Carpio* (Vinodhini and Narayana, 2009) and in the kidney of *S. senegalensis* (Velma and Tchounwou, 2011; Oliva *et al.,* 2012), in *C. batracus* (Bhattacharya and Bhattacharya, 2007)

As proposed by Tsangaris *et al.* (2007), GPx is not only an important component of antioxidant defence system but its response is known to be accompanied by the action of other anti oxidants (GST) and Scavenger (GSH) molecules. GST is one of the major phase II, GSH-dependent ROS- electrophilic xenobiotic detoxifying enzyme (Comakli *et al.,* 2011) by making the xenobiotic chemicals more hydrophilic for transportation or excretion. When severe oxidative damage prevents the primary antioxidants from functioning GST can still remove the harmful substance, allowing the cell to regain homeostasis (Perl-Treves and Perl, 2002). In animals the toxicant conjugate is marked for excretion, GST is therefore considered a 'detoxification enzyme' rather than a traditional antioxidant (Adeyemi, 2014; Mani *et al.,* 2014)**.** The elevated levels of GST in the present studies indicate the shift towards a detoxification mechanism under agro-chemical exposure. There is more GST activity in hepatic tissue compared to kidney and gills, which is due to effective role of liver in agrochemical detoxification (Wassmur *et al.,* 2010; Haluzová *et al.,* 2011). The increase in the activity of GST reported in the present study indicates the biotransformation pathway used a protective response in fish towards exposure to an oxidative stress induced by agro-chemicals (Wengu *et al.,* 2009; Dabas *et al.,* 2012 and Anushia *et al.,* 2012).

Among the enzymes that compromise the defence system against toxicity also includes superoxide dismutase (SOD) and catalase (CAT) (Otitoju, 2005). Superoxide dismutase, the first enzyme in the line of antioxidant defense, responsible for catalyzing the conversion of the superoxide ions into water and molecular oxygen via catalase. Antioxidant enzymes are used by the organisms as natural endogeneous protection against the generation of ROS (Metwalli and El-Megd, 2002). SOD catalyses the destruction (dismutation) of superoxide free radicals produced during oxidation of pesticide (Otitoju and Onwurah, 2007). The action of SOD therefore results in the protection of the biological integrity of cells and tissues against the harmful effects of superoxide free radicals (Van der Oost *et al*., 2003). To ameliorate the damage caused by the hydroxyl radicals formed from superoxide radical and hydrogen peroxide, organisms have evolved mechanisms to regulate the concentrations of the two reactants. The present study revealed that SOD and CAT activities in the liver, kidney and gills of *O.mossambicus* and *L.rohita* exposed to agrochemicals increased significantly. Thus, the induction of SOD and CAT may be a physiological adaptation for the elimination of ROS generation (Gad, 2011). As reported by (Sadekarpawar *et al.,* 2014), an increased in SOD is followed by a parallel increase in CAT, since both enzymes are linked functionally and occur in tandem. Similar results have been observed in the *Sparus aurata*, *Oreochromis mossambicus*, *Labeo rohita* and *Carassus auratus* (Gull *et al.,* 2004; Zhang *et al.,* 2004; Sun *et al.,* 2006; Correia et al., 2007; Sivaperumal, 2008; Zaidi and Soltani, 2010). Considering the results for each tissue, it was found that the liver showed the highest SOD and CAT antioxidant activity compared to kidney and gills. Both enzymes appeared to have an important role in combating the generation of superoxide radical ($O2^-$) and hydrogen peroxide (H_2O_2) from the intense metabolic activity characteristic of liver.

All the major biomolecules like lipids, proteins, and nucleic acids may be attacked by free radicals, but lipids are probably the most susceptible to peroxidative damage (LPO) (Ray and Akhtar, 2002). LPO has been identified as one of the basic deteriorative reactions in cellular mechanisms of the agro-chemical induced OS in fresh water fishes (Vardharajan, 2010). A dose dependent increase in the level of LPO was observed in liver, kidney and gills of the fish exposed to IMI and CZ. The elevated levels of LPO in the gills and livers of in response to 21 days of exposure agrochemicals that were observed during the present study suggest that production of ROS is increased, which could be associated with the metabolism of the agrochemicals leading to the peroxidation of membrane lipids in the tissues. Researchers have reported previously LPO induction by pesticides such as alachlor (Peebua *et a.l*, 2007), malathion (Chandra 2008), and butachlor (Farombi *et al.* 2008) in fishes. Our results are also corroborated with previous studies reported by other investigators (Oruc, 2010; Kavitha and Rao, 2008; Sharbidre *et al.,* 2011; Lopez-Lopez *et al.,* 2011). The impairment of enzymatic antioxidant system can facilitate the accumulation of free radicals that might be responsible for increased lipid peroxidation with agrochemical exposure.

CONCLUSION

Thus, from the present study it can be concluded that the response of antioxidant enzymes (SOD, CAT, GPx, and GST) and non-enzymatic antioxidant/scavengers (AA and GSH) confirmed that the teleosts are under severe oxidative stress. The enzymatic and the non-enzymatic antioxidant machinery are interacting in a concerted manner to eliminate ROS and prevent damage to cellular components. This suggests that IMI and CZ at levels below median lethal concentration are capable of causing oxidative damage in *O. mossambicus* and *L. rohita*.Nevertheless, concerted efforts in reducing the use of agrochemicals and

implementing natural remedies for pest encroachment through organic farming can help in resolving the problems of agrochemical pollution.

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