International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 6, Issue 5, pp: 248-255, 2021
https://doi.org/10.55126/ijzab.2021.v06.i05.023
http://www.ijzab.com

Research Article

ENZYMATIC CHANGES DURING LETHAL AND SUBLETHAL EXPOSURES OF GLYPHOSATE (41% SL) ON FRESHWATER FISH *CIRRHINUS MRIGALA*

[1*]A. Rajeswari and [2]K. Sunita

Department of Zoology & Aquaculture, Acharya Nagarjuna University, Nagarjuna Nagar 522510, Guntur, Andhra Pradesh, India

Article History: Received 3rd November 2021; Accepted 17th November 2021; Published 18th November 2021

ABSTRACT

The lethal and sublethal effect of the Glyphosate (41% SL) on certain enzymes in tissue such as AAT (Aspartate Amino Transferase), ALAT (Alanine Amino Transferase), LDH (Lactate Dehydrogenase) and ACP (Acid Phosphatase) of the freshwater fish, *Cirrhinus mrigala* during and after the cessation of the exposure were observed. Ten fish were exposed chronically to lethal and sublethal concentrations for a period of 4 days were sacrificed and tissues such as brain, gill, liver, kidney and muscle were analysed for biochemical parameters and remaining five fish were maintained in clean well water for a period of seven days without test substance being spilled in the water and its tissues were collected after reversal period for biochemical analysis. Marked changes were observed in the biochemical parameters of the fish exposed and after the cessation of the exposure

Keywords: Glyphosate toxicity, Aspartate Amino Transferase, Alanine Amino Transferase, Acid Phosphatase.

INTRODUCTION

Rapid industrialization and urbanization in the last few decades, with the concomitant growth in population, had taken a toll on the natural resources. Several anthropogenic activities like pollution by toxic substances through pesticides or heavy metals on regional or global scale results in climate change. Large-scale mortality of living organisms' most important wildlife such as sea mammals and expanding threat to human health, i.e., chronic respiratory diseases, cancer, damage to several major organs like the brain, lungs, kidneys are being witnessed in the recent years as a result of anthropogenic perturbations. Glyphosate was historically classified as a low toxicity herbicide, meaning that it was considered safe at environmentally realistic concentrations. (Borggaard & Gimsing, 2008) has been recently challenged by laboratory evidence showing that, even at concentrations below regulatory limits, glyphosate can have damaging effects on human cells, and by law suits in the US and the threat of ban in Europe. The latter is true not only in relation to glyphosate, but to most pesticides currently used, even though some measurable level of pesticides is found in the bodies of the vast majority of people in Western countries (Economist, 2016; Hakim, 2017; Landrigan, 2018; Mesnage *et al*., 2012). Only a few occupational biomonitoring exposure studies involving glyphosate have assessed exposure in the agricultural (Curwin *et al*., 2007; Mesnage *et al*., 2015) and horticultural sectors (Connolly *et al*., 2018). European environmental biomonitoring studies have identified low levels of glyphosate exposure in the general public (Connolly *et al*., 2017; Conrad *et al*., 2017; Krüger *et al*., 2014). Studies have identified dermal absorption to be the primary route of pesticide exposure, accounting for up to 99.9% of total exposure (Aprea *et al*., 2004; Flack *et al*., 2008; Vitali *et al*., 2009), with inhalation exposure only accounting for 1% of total exposure.

Thus the Glyphosate (41% SL) can show its effect on biochemical parameters of the freshwater fish, *Cirrhinus mrigala* as well on metabolic enzymes such as Aspartate Amino Transferase (AAT), Alanine Amino Transferase (ALAT), Lactate dehydrogenase (LDH) etc. *Cirrhinus mrigala* is one of the major carp species cultivated in India

*Corresponding Author: A. Rajeswari, Department of Zoology & Aquaculture, Acharya Nagarjuna University, Nagarjuna Nagar 522510, Guntur, Andhra Pradesh, India Email: rajiaplus@gmail.com.

and hence chosen as a model system to study the effects of glyphosate at lethal and sublethal concentrations on certain enzymes.

MATERIALS AND METHODS

The test fish *Cirrhinus mrigala* with a size range of 5-6 ± $^1/_2$ cm, irrespective of their sex have been chosen as the test organism in the present study. The freshwater fish were brought from a local fish farm and acclimatized to laboratory conditions for one week. Ground nut cake and rice bran was used as feed to the fish during the period of acclimatization. Fishes were exposed to static lethal and sublethal concentration ($1/10^{th}$ of LC_{50} value for 96 h) glyphosate for 96 hours or for 4 days.

Experiments were conducted to determine the toxicity of glyphosate in various concentrations within the static method. The data on the mortality rate of fish was recorded. The dead fish were removed immediately. The toxic tests were conducted to choose the mortality range from 10% to 90% for 4 days in static method. During the whole experiment, a suitable control was also maintained to nullify any other effects that likely to affect the fish. Then the fishes were scarified immediately and isolated fresh (wet) tissue of vital organs such as brain, gill, liver, kidney and muscle were taken for biochemical estimation of LDH, ACP, AAT and ALAT.

Estimation of Lactate Dehydrogenase (LDH) Activity

The Lactate Dehydrogenase activity (LDH) was estimated by the method of (Reddy, Murthy, Venkateswarlu, & Rao, 1985). Two percent homogenates of the tissue were prepared in 0.25 M ice-cold sucrose solution and centrifuged at 1000 rpm for 15 minutes. The supernatant served as the enzyme source. The reaction mixture of 2 ml contains 0.5 ml of lithium lactate, 0.5 ml of phosphate buffer, 0.2 ml of INT [(2-p-idophenol-3-(P-nitrophenyl)-5-(phenyl tetrazolium chloride)] and 0.2 ml of Nicotinamide Adenine Dinucleotide (NAD) and 0.6 ml of supernatant. The reaction mixture was incubated at 37ºC for 30 minutes stopped the reaction by adding 5 ml of acetic acid. Zero time controls were maintained by adding 5 ml of acetic acid prior to the addition of homogenate. The formazan formed was extracted overnight in 5 ml of cold toluene. The intensity of colour developed was read at 495 nm against a reagent blank in a spectrophotometer. The activity was expressed as moles of formazan formed/mg protein/hour.

Estimation of Acid Phosphatase (ACP) Activity

The activity of acid phosphatase was estimated by the method of (Bodansky, Hallman, & Bonoff, 1932). Two percent homogenates of the tissue were prepared in 0.25M ice cold sucrose solution and centrifuged at 1000 rpm for 15 minutes. The supernatant served as the enzyme source. The reaction mixture of 1.5 ml contains 1 ml of phosphate buffer (pH 5.3), 0.1 ml α-napthyl phosphate, Fast Red TR 0.1 ml and tartrate 0.2 ml. The contents were incubated at 37ºC for 30 minutes. In acidic pH of buffer system, acid phosphatase hydrolyses α-napthyl phosphate to α-napathal and phosphate. The α-napthal is then coupled with diazotized fast red TR to form a diazo dye which has strong absorbance at 405 nm. The addition of L-tartrate inhibits the reaction. Zero time controls were maintained by adding 5 ml of L-tartrate prior to the addition of homogenate. The intensity of colour developed was read at 405 nm against a reagent blank in a spectrophotometer. The activity was expressed as µg pi/g protein/hour.

Estimation of AAT activity

The reaction mixture of 1.5 ml contains 1 ml phosphate buffer (pH 7.4), 0.1 ml of L-alanine, 0.1 ml of -ketoglutarate and 0.3 ml of supernatant as enzyme source. The contents were incubated at 37ºC for 30 minutes. The reaction was stopped by the addition of 1 ml of 2, 4-dinitrophenyl hydrazine solutions. After 20 minutes, 10 ml of 0.4 N Sodium Hydroxide was added and the colour developed was read at 545 nm in a spectrophotometer against a reagent blank. The enzyme activity was expressed as moles of pyruvate formed/mg protein/hour.

Estimation of ALAT activity

The reaction mixture of 1.5 ml contains: 1 ml of phosphate buffer (pH 7.4), 0.1 ml of L-aspartate (L-Aspartic acid), 0.1 ml of ketoglutaric acid and 0.3 ml of supernatant as enzyme source. The reaction mixture was incubated at 37ºC for 30 minutes. The reaction was stopped by adding 1 ml of 2, 4-dinitrophenyl hydrazine solution prepared in 0.1 N HCl and was allowed to stand for 20 minutes at room temperature. The rest of the details were the same as for alanine aminotransferase. The activity levels were expressed as moles of pyruvate formed/mg protein/hour.

RESULTS AND DISCUSSION

The calculated mean values of LDH activity along with standard deviation values and the percent change over control are represented in Table 1 during 96 hours Glyphosate exposure. In control fish of *C. mrigala*, the values of LDH activity in different tissues are in the order of: Muscle > Gill > Liver > Brain > Kidney. In the fish reared as control for 96 hours, the LDH activity was the highest in muscle (0.2072 µ moles of formazan/mg of protein/h), followed by gill (0.1572 µ moles of formazan/mg of protein/h) and liver (0.1092 µ moles of formazan/mg of protein/h). Moderate values were observed in brain (0.0934 µ moles of formazan/mg of protein/h) and very low in kidney (0.0817 µ moles of formazan/mg of protein/h) respectively. The total LDH content significantly increased ($p < 0.05$) during sublethal and lethal exposures of Glyphosate (41% SL) when compared to the control fish group. The percent depletion of glycogen in the test fish *C. mrigala* under 41% SL of Glyphosate exposure is in the following order (Table 1).Glyphosate Sublethal for 96 hours: Kidney > Liver > Brain > Muscle > Gill. Glyphosate Lethal for 96 hours: Kidney > Brain > Liver > Muscle > Gill.

Table 1. Change in the specific activity levels of Lactate Dehydrogenase (LDH) (µ moles of formazan/mg of protein/hr) and % change over to control in different tissue of *C. mrigala* on exposure to sublethal and lethal concentration of glyphosate (41% SL) 96 hours.

Tissue	Control(M±SD)	Sublethal (M±SD)	% Change	Lethal (M±SD)	% Change
Gill	0.1572±0.007	0.1742±0.05	10.814	0.1942±0.06	23.536
Brain	0.0934±0.003	0.1172±0.013	25.481	0.1374±0.014	47.109
Liver	0.1092±0.004	0.1372±0.032	25.641	0.1573±0.08	44.047
Kidney	0.0817±0.042	0.1073±0.003	31.334	0.1244±0.06	52.264
Muscle	0.2072±0.09	0.2319±0.06	11.920	0.2974±0.083	43.532

M±SD = Mean of 5 values± Standard Deviation, Values are significant at $p < 0.05$.

Table 2. Change in the specific activity levels of Aspartate amino transferase (AAT) (µ moles of pyruvate formed/mg of protein/h) and % change over to control in different tissue of *C. mrigala* on exposure to sublethal and lethal concentration of glyphosate (41% SL) 96 h.

Tissue	Control (M±SD)	Sublethal (M±SD)	% Change	Lethal (M±SD)	% Change
Gill	8.983±0.85	10.976±0.87	22.186	12.364±0.93	37.637
Brain	6.643±0.13	7.917±0.39	19.178	8.837±1.07	33.027
Liver	14.373±1.07	20.876±1.68	45.234	22.473±1.35	56.344
Kidney	5.139±0.98	6.609±0.56	28.604	7.367±1.16	43.354
Muscle	9.573±0.62	12.983±1.07	35.621	14.987±0.50	56.554

M±SD = Mean of 5 values± Standard Deviation, Values are significant at $p < 0.05$.

Table 3. Change in the specific activity levels of Alanine amino transferase (ALAT) (µ moles of pyruvate formed /mg protein/hr) and % change over to control in different tissue of *C. mrigala* on exposure to sublethal and lethal concentration of glyphosate (41% SL) 96 h.

Tissue	Control (M±SD)	Sublethal (M±SD)	% Change	Lethal (M±SD)	% Change
Gill	3. 217± 092	4.317±0.94	34.19	5. 345 ± 0.68	66.14
Brain	2. 316 ± 0.62	3.437±0.72	48.40	3. 138 ± 1.16	35.49
Liver	7. 123 ± 1.39	8.142±1.40	14.305	12. 165 ± 0.96	70.784
Kidney	4. 427 ± 0.72	5.527±0.73	24.84	6. 022 ± 1.22	36.02
Muscle	4. 782 ± 1.35	5.784±1.36	20.95	7. 014± 1.71	46.675

M±SD = Mean of 5 values± Standard Deviation, Values are significant at $p < 0.05$.

Table 4. Change in the specific activity levels of Acid Phosphatase activity (ACP) (mg pi/g of protein/h) and % change over to control in different tissue of *C. mrigala* on exposure to sublethal and lethal concentration of glyphosate (41% SL) 96 h.

Tissue	Control (M±SD)	Sublethal (M±SD)	% Change	Lethal (M±SD)	% Change
Gill	12.36±1.42	14.97±1.32	21.116	17.436±1.81	41.067
Brain	8.37±0.92	10.34±1.36	15.401	12.153±1.76	35.636
Liver	14.52±0.96	19.37±1.82	33.402	21.836±1.32	50.385
Kidney	8.96±1.36	12.29±1.52	46.833	13.672±1.35	63.345
Muscle	9.68±0.82	11.98±0.91	23.760	13.875±1.64	43.336

M±SD = Mean of 5 values± Standard Deviation, Values are significant at $p < 0.05$.

In the present study, the levels of LDH activity was found to be increased in all fish tissues of *C. mrigala* exposed to glyphosate at sublethal and lethal concentration for 96 h when compared to the control fish group. In the present analysis, the LDH activity levels increased in both nervous (brain) and non-nervous (kidney, muscle, liver and gill) organs of the fish, *C. mrigala* exposed to the toxicity of Glyphosate. The elevation in LDH activity levels found to be more in lethal than sublethal exposure for 96 hours. In the present investigation, it was observed that the activity of LDH was highly elevated by following Glyphosate exposures, which indicating increased anaerobic respiration to meet the energy demands where aerobic oxidation is lowered. Lactate dehydrogenase (LDH) converts the lactate to pyruvate and it plays a very important role in carbohydrate metabolism. The LDH activity depends on especially its five isoenzymes and the activity changes under the influence of pathological conditions (Rodwell *et al*., 1983). Tilak *et al*., (2009) found that the activity of LDH increased in freshwater fish *Channa punctatus* (Bloch) exposed to lethal and sublethal concentrations of a chloroacetanilide herbicide Alachlor. Increase in LDH levels was found in liver and muscles of propiconazole exposed *Channa punctatus* (Pallavi & Ajay, 2013). Blahova *et al*., (2014) noticed statistically significant alterations in LDH of atrazine treated common carp (*Cyprinus carpio*). Observed elevated levels of LDH in *Channa punctatus* exposed to methyl parathion 50% EC.

Thus, the observed increased LDH can be interpreted as a shift in the respiratory metabolism from aerobic to anaerobic in order to meet the enhanced energy demand under the toxic stress (Kamalaveni *et al*., 2003). Subsequently LDH enzyme play a major role in fish energy metabolism than it does in mammals, particularly in conditions of pesticide stress when high energy levels may be required in a short period of time (Das *et al*., 2004; Orrego *et al*., 2011) reported that, with increasing ammonia concentration, there was a progressive increase in LDH activity in gill, liver, kidney and brain of the exposed fingerlings of *C. mrigala* and increased the rate of glycolysis. Similar observations were noticed by various researchers in increasing LDH activity in kidney, liver, brain and muscle of *L. rohita* (Desai, 2006; Tilak *et al*., 2004). In the present study, it was observed that the activity of LDH in the fish tissues of *C. mrigala* under exposure to sublethal and lethal concentrations of Glyphosate. This shows that, the anaerobic respiration induced and aerobic respiration inhibited so as to meet the increased metabolic stress to overcome the toxic stress. According to our investigation Glyphosate exhibited more toxicity during the experiment. The changes in the LDH activity levels were significant at $p < 0.05$ in all the tissues of fish during 96 h sublethal exposures respectively.

The calculated mean values of AAT activity along with standard deviation values and the percent change over control are represented in Table 2 during 96 hours Glyphosate exposure. In control fish of *C. mrigala*, the values of AAT activity in different tissues are in the order of Liver > Muscle > Gill > Brain > Kidney. In the fish reared as control for 96 hours, the AAT activity was the highest in liver (14.373 µ moles of pyruvate formed/mg of protein/h), followed by muscle (9.573 µ moles of pyruvate formed/mg of protein/h) and gill (8,983 µ moles of pyruvate formed/mg of protein/h). Moderate values were observed in brain (6.643 µ moles of pyruvate formed/mg of protein/h) and very low in kidney (5.139 µ moles of pyruvate formed/mg of protein/h) respectively. The total AAT content significantly increased ($p < 0.05$) during both sublethal and lethal exposures of Glyphosate (41% SL) when compared to the control fish group. The percent depletion of glycogen in the test fish *C. mrigala* under 41% SL of Glyphosate exposure is in the following order (Table 2), Glyphosate Sublethal for 96 hours: Liver > Muscle > kidney > Gill > Brain Glyphosate Lethal for 96 hours: Muscle > liver > Kidney > Gill > Brain.

In the present study, the levels of AAT activity was found to be increased in tissues of *C. mrigala* on administration of sublethal and lethal doses of Glyphosate for 96 hours when compared to control group. In the present investigation, the data on AAT activity levels elevated in both the nervous (brain) and non-nervous (gill, liver, muscle and kidney) organs of the fish *C. mrigala* exposed to sublethal and lethal toxicity of both the pesticides. The elevation in Asparate Amino Transferase activity levels found to be more in lethal than sublethal exposure for 96 hours. The calculated mean values of ALAT activity along with standard deviation values and the percent change over control are represented in Table 3 during 96 hours Glyphosate exposure. In control fish of *C. mrigala*, the values of ALAT activity in different tissues are in the order of Liver > Muscle > Kidney > Gill > Brain. In the fish reared as control for 96 hours, the ALAT activity was the highest in liver (7.123 µ moles of pyruvate formed /mg of protein/h), followed by muscle (4.782 µ moles of pyruvate formed/mg of protein/h) and kidney (4.427 µ moles of pyruvate formed /mg of protein/h). Moderate values were observed in gill (3.217 µ moles of pyruvate formed /mg of protein/ h), very low in brain (2.316 µ moles of pyruvate formed /mg of protein/ h) respectively. The total ALAT content significantly increased ($p < 0.05$) during both sublethal and lethal exposures of Glyphosate (41% SL) when compared to the control fish group. The percent depletion of glycogen in the test fish *C. mrigala* under 41% SL of Glyphosate exposure is in the following order (Table 3). Glyphosate Sublethal for 96 hours: Liver > Muscle > Gill > Kidney > Brain. Glyphosate Lethal for 96 hours: Liver > Muscle > Gill > Kidney > Brain.

In the present study, the levels of ALAT activity was found to be increased in tissues of *C. mrigala* on administration of sublethal and lethal doses of Glyphosate for 96 hours when compared to control group. In the present investigation, the data on ALAT activity levels elevated in the nervous (brain) and non-nervous (gill, liver, muscle and kidney) organs of the fish *C. mrigala* exposed to lethal and sublethal toxicity of the pesticides. The elevation in Alanine Amino Transferase activity levels found to be more in lethal than sublethal exposure for 96

hours. The changes in the levels of AAT and ALAT were studied in different tissue of gill, brain, liver, kidney and muscle in the test fish *C. mrigala* under sublethal and lethal concentrations of glyphosate after 24, 48, 72 and 96 hours of exposure. The increase of AAT activity provides the oxaloacetate required for the gluconeogenesis pathway to meet the additional supply of glucose for the production of energy under reduced phase of oxidative metabolism. Elevation in the levels of AAT and ALAT in different tissue of liver, muscle, gill, kidney and brain of the fish *C. mrigala* can be considered as a response to the stress induced by glyphosate to generate ketoacids like α-ketoglutarate and oxaloacetate for contributing to gluconeogenesis and or energy production necessary to meet the excess energy demand under the toxic manifestations. The depletion of proteins under the stress of glyphosate toxicity observed in different tissue of *C. mrigala* indicates the proteolysis, prompting the suggestion that the proteins were utilized to meet the excess energy demands imposed by the toxic stress. The alterations in the levels of activity of aminotransferases induced by the pesticide glyphosate clearly indicate that the stress brings about the metabolic reorientation in the tissue by raising energy resources through transaminase systems.

AAT and ALAT are placed in both mitochondrial and cytosol fractions of the cell. A close relation seems to exist between the mitochondrial integrity and transaminase levels and any modification in the organization of mitochondria is bound to alter the enzyme systems associated with it. The activity of AAT, ALAT enzymes increased in alachlor treated *Channa punctatus* (Bloch) (Tilak *et al*., 2009). Three Indian freshwater fishes *Anabas testudineus, Heteropneustes fossilis* and *Oreochromis niloticus* were exposed to almix and observed significant elevation in the levels of ALAT and AAT. The activity of AAT and ALAT were found to be elevated in methyl parathion 50% EC exposed fish, *Channa punctatus*. (Yildirim *et al*., 2006) have reported an increase in AAT and ALAT enzyme activities in gills, liver and kidney and have proposed that elevated enzyme activity is with the intension to increase the role of proteins for energy production during stress. Similar elevation in aminotransferases also have been reported by peer researchers (Agbor *et al*., 2011; Gabriel *et al*., 2012; Velmurugan *et al*., 2008), which is due to the increased utilization of amino acids for energy synthesis as a consequence of this, fish suffers from toxic stress and energy crisis.

During the experiment, it was observed that the reduction of proteins under the stressful condition due to Glyphosate toxicity observed in different tissues of fish *C. mrigala* indicates proteolysis, prompting the suggestion that the proteins were utilized to meet the excess energy demands imposed by the toxic stress. Thus, the changes in the activity levels of aminotransferases induced by both the pesticides in experiment clearly indicated that the stress brings about the metabolic reorientation in the tissues by raising energy resources through transaminase systems. The statistical analysis shows significant increase ($p < 0.05$) in the AAT and ALAT activity levels in all the tissues except intestinal tissues of fish *C. mrigala* under Glyphosate toxicity. The calculated mean values of ACP activity along with standard deviation values and the percent change over control are represented in Table 4 during 96 hours Glyphosate exposure. In control fish of *C. mrigala*, the values of ACP activity in different tissues are in the order of Liver > Gill > Muscle > Kidney > Brain. In the fish reared as control for 96 hours, the ACP activity was the highest in liver (14.52 mg pi/g of protein/h), followed by gill (12.36 mg pi/g of protein/h) and muscle (9.68 mg pi/g of protein/h). Moderate values were observed kidney (8.96 mg pi/g of protein/h), very low in brain (8.37 mg pi/g of protein/h) respectively. The total ACP content significantly increased ($p < 0.05$) during both sublethal and lethal exposures of Glyphosate (41% SL) when compared to the control fish group. The percent depletion of glycogen in the test fish *C. mrigala* under 41% SL of Glyphosate exposure is in the following order (Table 4). Glyphosate Sublethal for 96 hours: Kidney > Liver > Muscle > Gill > Brain Glyphosate Lethal for 96 hours: Kidney > Liver > Muscle > Gill > Brain.

In the present study, the levels of ACP activity was found to be increased in all fish tissues of *C. mrigala* (muscle, liver, brain, gill and kidney) exposed to glyphosate at sublethal and lethal concentration for 96 h when compared to the control fish group. In the present investigation, the ACP activity level increases in all the tissues of fish *C. mrigala* exposed to toxicity of Glyphosate during sublethal and lethal exposure. The elevation in ACP level was found to be high during 96 h sublethal and lethal exposure periods. The ACP is a hydrolytic enzyme released by lysosomes for the hydrolysis of and the raise in its activity is probably related to the cellular damage. The increased ACP activity seems to resulted from enhanced enzyme turn over under pesticide stress. Venkatesan *et al*., (2012) recorded increased values of ACP in different organs like gill, liver and kidney during atrazine exposure to fish *Cyprinus carpio.* Magar & Shaikh, (2012) reported significant decrease in acid phosphatase activity of liver and muscle in malathion treated group compared with control. The activity of ACP in treated fishes were significantly reduced ($p < 0.05$) in response to treatment of alphamethrin as compared to control in zebrafish, *Danio rerio* (Israel *et al*., 2014). Marked increase in ACP activity was observed in freshwater crab, Paratelphusa (Barytelphusa) jacquemontii exposed to malathion (Patil *et al*., 2014). Decreased ACP values were recorded in the liver, muscle and brain of freshwater fish *Cyprinus carpio* exposed to lindane (Israel *et al*.1882). Vasantharaja *et al*., (2014) noticed decreased values of ACP in cypermethrin treated freshwater fish *C. mrighala.*

In the present study, the mean value of ACP activity in the increased during the 96 h exposure. This increased activity was due to the cellular damage caused by hepatotoxins or a response to overcome toxicity of Glyphosate. The significant difference in phosphatases activities between the control and experimental groups of fish species might be considered due to the damage of

hepatic tissue with dysfunctions of organs. The elevation in ACP activity proposes an increase in the lysosomal mobilization and cell necrosis due to the pesticide toxicity (Rao, 2006). The enzyme ACP activity elevation in brain tissue was described in stress-exposed *C. punctatus* (Naveed *et al*., 2011). The sub-acute exposure pesticide chlorpyrifos revealed increased activity of ACP content in the liver and kidney tissues of *Gumbusia affinis*, and ACP activity is a conventional indicator of liver damage in the fish (Khan & Sharma, 2012). Dose dependent and significant increase in the activity of acid phosphatase may be attributed to the hepatic and renal damage (Sreenivasan *et al*., 2010).

CONCLUSION

The results of the present experiment are in correlation with the previous work done on various fish species exposed to different toxicants where ACP levels were increased (Barse *et al*., 2006; Sharma, 2014). The statistical analysis indicated the significant increase ($p < 0.05$) in ACP activity levels in all the tissues but the increase is not significant ($p < 0.05$) in the tissues during 96 h exposure.

ACKNOWLEDGEMENTS

The authors thank the Head of the Department of Zoology, Acharya Nagarjuna University for providing necessary facilities, and also thank UGC for providing equipment to carry out this work under Special Assistance Program (UGC-SAP-DRS-Phase.III

REFERENCES

Agbor, G. A., Taga, I., Nguindex, D. R., Zaidi, M. A., Lehman, L., Altosaar, I., & Ngogang, J. Y. (2011). Effect of iodine supplementation on antioxidant status of normal and alloxan monohydrate in toxicated rats. *International Journal of Pharmacology*, *7*(6), 726-731.

Aprea, C., Terenzoni, B., De Angelis, V., Sciarra, G., Lunghini, L., Borzacchi, G., Salvan, A. (2004). Evaluation of skin and respiratory doses and urinary excretion of alkylphosphates in workers exposed to dimethoate during treatment of olive trees. *Archives of Environmental Contamination and Toxicology, 48*(1), 127-134.

Barse, A., Chakrabarti, T., Ghosh, T., Pal, A., & Jadhao, S. (2006). One-tenth dose of LC50 of 4-tert-butylphenol causes endocrine disruption and metabolic changes in Cyprinus carpio. *Pesticide Biochemistry and Physiology*, *86*(3), 172-179.

Blahova, J., Modra, H., Sevcikova, M., Marsalek, P., Zelnickova, L., Skoric, M., & Svobodova, Z. (2014). Evaluation of biochemical, haematological, and histopathological responses and recovery ability of common carp (Cyprinus carpio L.) after acute exposure to atrazine herbicide. *BioMed Research International, 2014*.

Bodansky, A., Hallman, L., & Bonoff, R. (1932). Phosphatase studies. 1. Determination of inorganic phosphate. Beer's law and interfering substances in the KuttnerLichtenstein method. *Journal of Biological Chemistry*, *99*, 197-206.

Borggaard, O. K., & Gimsing, A. L. (2008). Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. *Pest Management Science: Formerly Pesticide Science, 64*(4), 441-456.

Connolly, A., Jones, K., Galea, K. S., Basinas, I., Kenny, L., McGowan, P., & Coggins, M. (2017). Exposure assessment using human biomonitoring for glyphosate and fluroxypyr users in amenity horticulture. *International journal of Hygiene and Environmental Health, 220*(6), 1064-1073.

Connolly, A., Leahy, M., Jones, K., Kenny, L., & Coggins, M. A. (2018). Glyphosate in Irish adults A pilot study in 2017. *Environmental Research, 165*, 235-236.

Conrad, A., Schröter-Kermani, C., Hoppe, H.-W., Rüther, M., Pieper, S., & Kolossa-Gehring, M. (2017). Glyphosate in German adults–Time trend (2001 to 2015) of human exposure to a widely used herbicide. *International Journal of Hygiene and Environmental Health, 220*(1), 8-16.

Curwin, B. D., Hein, M. J., Sanderson, W. T., Striley, C., Heederik, D., Kromhout, H., Alavanja, M. C. (2007). Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in Iowa. *The Annals of Occupational Hygiene, 51*(1), 53-65.

Das, P. C., Ayyappan, S., Jena, J., & Das, B. (2004). Acute toxicity of ammonia and its sub-lethal effects on selected haematological and enzymatic parameters of mrigal, *Cirrhinus mrigala* (Hamilton). *Aquaculture Research, 35*(2), 134-143.

Desai, B. N. (2006). Effects of Agro-Chemicals on the physiological stress on teleost fish. Ph.D. Thesis. The Maharaja Sayajirao University of Baroda, Vadodara, India

Economist, T. (2016). Fog of uncertainty-regulators are arguing over the safety of glyphosate, the world's top weedkiller. *March, 3*, 2016.

Flack, S., Goktepe, I., Ball, L. M., & Nylander-French, L. A. (2008). Development and application of quantitative methods for monitoring dermal and inhalation exposure to propiconazole. *Journal of Environmental Monitoring, 10*(3), 336-344.

Gabriel, U., Akinrotimi, O., & Ariweriokuma, V. (2012). Changes in metabolic enzymes activities in selected organs and tissue of Clarias gariepinus exposed to cypermethrin. *Journal of Chemical Engineering, 1*(1), 25-30.

Hakim, D. (2017). Monsanto weed killer Roundup faces new doubts on safety in unsealed documents. *New York Time*.

Israel, M. A., Chidobem, J. I., & Onyemaechi, E. C. Oxidative stress, haematology and histology biomarkers of African clariid catfish clarias gariepinus (Burchell 1822) to glyphosate-based herbicide GBHG,'Glycot'.

Kamalaveni, K., Gopal, V., Sampson, U., & Aruna, D. (2003). Recycling and utilization of metabolic wastes for energy production is an index of biochemical adaptation of fish under environmental pollution stress. *Environmental Monitoring and Assessment, 86*(3), 255-264.

Khan, S., & Sharma, N. (2012). A study on enzymes acid phosphatase and alkaline phosphatase in the liver & kidney of fish Gambusia affinis exposed to the chlorpyrifos, an organophosphate. *International Journal of Pharmaceutical Sciences Review and Research, 13*(1), 88-90.

Krüger, M., Schledorn, P., Schrödl, W., Hoppe, H.-W., Lutz, W., & Shehata, A. A. (2014). Detection of glyphosate residues in animals and humans. *Journal of Environmental Anals Toxicolology, 4*(2), 1-5.

Landrigan, P. J. (2018). Pesticides and human reproduction. *JAMA Internal Medicine, 178*(1), 26-27.

Magar, R., & Shaikh, A. (2012). Biochemical changes in proteins and amino acids in Channa punctatus in responses to sub-lethal treatment with the insecticide malathion. *Trends in Life Sciences, 1*(3), 19-23.

Mesnage, R., Defarge, N., De Vendômois, J. S., & Seralini, G. (2015). Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. *Food and Chemical Toxicology, 84*, 133-153.

Mesnage, R., Moesch, C., Grand, R., Lauthier, G., Vendômois, J., Gress, S., & Séralini, G. (2012). Glyphosate exposure in a farmer's family. *Journal of Environment Protection, 3*(9), 1001.

Naveed, A., Janaiah, C., & Venkateshwarlu, P. (2011). The effects of lihocin toxicity on protein metabolism of the fresh water edible fish, *Channa punctatus* (Bloch). *Journal of Toxicology and Environmental Health Sciences, 3*(1), 018-023.

Orrego, R., Guchardi, J., Beyger, L., Krause, R., & Holdway, D. (2011). Comparative embryotoxicity of pulp mill extracts in rainbow trout (*Oncorhynchus mykiss*), American flagfish (Jordanella floridae) and Japanese medaka (*Oryzias latipes*). *Aquatic Toxicology, 104*(3-4), 299-307.

Pallavi, S., & Ajay, S. (2013). Study of in-vivo effects caused by metabolites (1, 2, 4-trizole alanine) of steroid-inhibitor fungicide on aquatic life (fish). *Journal of Aquaculture Research and Development, 4*(4).

Patil, V., Kadam, G., & Raut, P. (2014). Enzymological alterations produced by chronic malathion exposure in freshwater crab, paratelphusa (barytelphusa) jacquemontii. *International Journal of Science, Environment and Technology, 3*(1), 303-313.

Rao, J. V. (2006). Sublethal effects of an organophosphorus insecticide (RPR-II) on biochemical parameters of tilapia, Oreochromis mossambicus. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 143*(4), 492-498.

Reddy, M. S., Murthy, B. N., Venkateswarlu, Y., & Rao, K. (1985). Toxicity of insecticide phosphamidon on tissue carbohydrate catabolism of penaeid prawn *Penaeus indicus* (H. Milne Edwards). *Journal of Scientific and Industrial Research*, 1, 206-207

Rodwell, V., Martin, D., Mayg, P., & Garnner, D. (1983). Harper's review of Biochemistry. *DW Martin*, 292-295.

Sharma, N. (2014). Assessment of the changes in some diagnostic enzymes in fish Gambusia affinis treated with pesticide chlorpyrifos. *International Journal of Research in Pharmcology and Pharmacotherapeutics, 3*(4), 205-210.

Sreenivasan, R., Moorthy, P. K., & Deecaraman, M. (2010). Variations in the enzyme activity of gills and hemolymph in cypermethrin, a pesticide treated freshwater female field crab, *Spiralothelphusa hydrodroma* (Herbst). *International Journal of Sustainable Agriculture, 2*(1), 10-15.

Tilak, K., Raju, P. W., & Butchiram, M. (2009). Effects of alachlor on biochemical parameters of the freshwater fish, *Channa punctatus* (Bloch). *Journal of Environmental Biology, 30*(3), 420-426.

Tilak, K., Veeraiah, K., & Rao, D. K. (2004). Toxicity and bioaccumulation of chlorpyrifos in Indian carp *Catla catla* (Hamilton), *Labeo rohita* (Hamilton), and *Cirrhinus mrigala* (Hamilton). *Bulletin of Environmental Contamination and Toxicology, 73*(5), 933-941.

Vasantharaja, C., Pugazhendy, K., & Meenambal, M. (2014). Studies on impact of cypermethrin on acid and alkaline phosphatase activity in the selected organs of freshwater fish *Cirrhinus mrigala* (Hamilton) and the protective effect of Cardiospermum halicacabum. *International Research Journal of Pharmaceutical, 5*(9), 667-670.

Velmurugan, B., Selvanayagam, M., Cengiz, E. I., & Uysal, E. (2008). Levels of transaminases, alkaline phosphatase, and protein in tissues of *Clarias gariepienus* fingerlings exposed to sublethal concentrations of cadmium chloride. *Environmental Toxicology: An International Journal, 23*(6), 672-678.

Venkatesan. S, K. P., C. Vasantharaja, M, Meenambal, D. Sangeetha, and Prabakaran, S. (2012). Impact of atrazine on acid and alkaline phosphatase activities and protective role of Spirulina in various organs of fish *Cyprinus carpio*. Linn. *International Journal Environmental Biology, 2*(1), 31-35.

Vitali, M., Protano, C., Del Monte, A., Ensabella, F., & Guidotti, M. (2009). Operative modalities and exposure to pesticides during open field treatments among a group of agricultural subcontractors. *Archives of Environmental Contamination and Toxicology, 57*(1), 193-202.

Yildirim, M. Z., Benlı, A. Ç. K., Selvı, M., Özkul, A., Erkoç, F., & Koçak, O. (2006). Acute toxicity, behavioral changes, and histopathological effects of deltamethrin on tissues (gills, liver, brain, spleen, kidney, muscle, skin) of Nile tilapia (*Oreochromis niloticus* L.) fingerlings. *Environmental Toxicology: An International Journal, 21*(6), 614-620.