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AMELIORATIVE POTENTIAL OF AQUEOUS EXTRACT OF *MORINGA OLEIFERA* LEAF AGAINST IMIDACLOPRID INDUCED HEPATOTOXICITY IN ZEBRA FISH, *DANIO RERIO*

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ABSTRACT: The current study was aimed to estimate the ameliorative potential of Moringa leaf extract (MLE) against the toxicity of Imidacloprid (IMC) on hepatic biomarkers in zebra fishes, *Danio rerio* after acute exposure. To analyze liver biomarkers, 20 fishes were randomly selected and divided into four groups. Group- I served as control, Group- II received MLE- 12 ml/l , Group III- treated with 24 h LC_{50} of IMC (0.423 ml/l), Group- IV received 0.423 ml/l of IMC + 12 ml/l of MLE. The same protocol was implemented by taking 96 hrs LC_{50} of IMC (0.270 ml/l) and MLE (10 ml/l). At the end of the experiment, liver was dissected out, homogenized, and centrifuged separately to collect supernatant for enzyme analysis. The activity of ALT, ALP, LDH, and GGT were increased in IMC exposed fishes after 24 and 96 h exposure periods, whereas protein contents were decreased. The AST activity was decreased after 24 h and increased after 96 h exposure period. Administration of MLE along with IMC, reversed the impact of IMC. These findings highlight that MLE have great importance in the amelioration of some biochemical disturbances associated with the acute toxicity of IMC.

INTRODUCTION: Over the last few decades, contamination of freshwater resources with chemical substances has become an issue of great attention. Broad-spectrum use of synthetic pesticides causes serious health problems and hazardous risk to the environment. A huge amount of pesticides that have deficient target specificity do not reach intentional target places; instead, they go to the aquatic environment. These pesticides affect aquatic non-target organisms, especially fishes. Fishes are quite sensitive to the wide range of pollutants and serve as the best bio-indicator to assess the toxicity of pesticides.

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Among the pesticides, Imidacloprid [1-6-chloro-3-pyridylmethyl)-N- nitroimidazolidin -2- ylidene-amine] is a widely used systemic chloronicotinyl insecticide for crop protection to control sucking insects, termites, tick, and mites. It acts as a neurotoxin for insects that attack their central nervous system, resulting in the impairment of nerve functions. Imidacloprid is considered to have toxicity to fishes altering their physiology and increasing death rate [1, 2].

The liver is the most important organ that performs vital functions in the regulation of physiological processes in the body. It is the main site of detoxification of chemicals. Liver damage is mainly caused due to excess consumption of alcohol, toxic chemicals, and infectious diseases. Several enzymes such as transaminases, phosphatases, dehydrogenases and oxidative enzymes are altered by pesticide exposure [3, 4, 5]. Exposure of Imidacloprid causes behavioral, physiological,

hematological, and biochemical changes in fishes [1, 6, 7]. The altered level of AST, ALT, ALP, LDH, GGT, and total protein contents were reported by many workers in different fish species after intoxication [3, 8, 9].

Pesticides undergo rapid redox cycling and may generate Reactive Oxygen Species (ROS) at a faster rate by altering the oxygen-free radical scavenging enzyme system. Excessive ROS promotes lipid peroxidation and causes cell damage [10]. It has been reported that exposure to pesticides can affect the balance between the generation of reactive oxygen species and antioxidant defense in fishes by several authors [11, 12, 13]. It has also been observed that a high concentration of Imidacloprid induced excessive production of ROS during stressed conditions in zebra fishes [14]. Naturally, most of the antioxidant compounds derived from several plant sources have the potential to reduce Reactive Oxygen Species. Polyphenolic compounds from a natural source can improve the antioxidant defense system in the body by scavenging free radicals [15, 16]. So the use of antioxidants is an important tool in obtaining, retaining, and restoring good health. *Moringa oleifera* (Moringaceae) is a multipurpose, medicinal, indigenous plant found in India. It is commonly known as "Miracle tree" or "Drumstick tree". It has several antioxidants such as caffeoylquinic acid, chlorogenic acid, and a rich combination of therapeutically active compounds such as Kaempferol, rhamnetin, rutin, quercitin, apigenin and also enriched in ascorbic acid, carotenoids, polyphenols, flavonoid glycosides, thiocarbamate glycosides, amino acids like methionine, lysine, cysteine, tryptophan [17, 18, 19].

Different parts of *Moringa oleifera* such as stem, roots, flowers, pods and leaves are being processed into medicines to cure serious diseases. The leaves of *Moringa oleifera* are highly nutritious rich in protein, minerals, vitamins, β-carotein, various polyphenolic compounds and natural antioxidants which have been utilized in medicine for the treatment of various human ailments [20]. It also contains acetone which is an effective remedy for malaria treatment [21]. The leaf extract was reported to prevent biochemical alterations, oxidative damage by enhancing the activities of antioxidant enzyme system, inhibiting the lipid peroxidation intensity and generation of free radicals [22]. Several works have been carried out to determine protective effect of *Moringa oleifera* leaf extract against heavy metal such as arsenic, cadmium and drugs like acetaminophen induced toxicity in rats [23, 24]. It has been reported that *Moringa oleifera* leaf extract helps in improving growth, and immunity in fishes [25]. The hepatoprotective role of *Moringa oleifera* has also been reported by several authors in rats and fishes [15, 26, 27].

Zebrafish has become the most popular experimental model because of the similarities in the sequenced genes to human beings [28]. Although, IMC has been widely used in recent times, data on its potential toxic effects on zebra fishes are insufficient. Further, the hepatoprotective role of MLE following Imidacloprid exposure in zebra fishes is also sparse. Therefore, the current study was carried out to determine the ameliorative potential of leaf extract of *Moringa oleifera* on hepatic biomarkers in Imidacloprid induced zebra fish, *Danio rerio.*

MATERIALS AND METHODS:

Collection and Acclimatization of Test Species: Zebra fishes, *Danio rerio* (weight 2-4g) were selected as a test organism for all the experimental work. These specimens were procured from aquarium shop of Jhansi district, U.P. India and carried in polythene bags to the laboratory, then shifted into well aerated glass aquarium. The fishes were treated to a prophylactic remedy by bathing in 0.1% potassium permanganate ($KMnO_4$) for 2 minutes to prevent cutaneous infections. They were allowed to acclimatize for 10-15 days under laboratory conditions before the commencement of the experiments. Temperature (25º-28º), dissolve oxygen (5.0-6.5mgl^{-1}), pH (7.2 ± 0.2), hardness (220mgl^{-1}) of water were maintained throughout the acclimatization. Fishes were fed with commercial food pellets. The water was changed and faecal matter and other waste products were siphoned off daily. The feeding was stopped 24h prior to acute toxicity bioassay.

All the experiments were carried out by keeping acclimatized fishes in rectangular glass aquaria size of 2′×1′×1′ separately according to groups. The application of animals for research work was granted by the Institutional Animal Ethical

Committee (CPCSEA) Govt. of India, New Delhi with Approval No.– BU/Pharm/ IAEC/March/2020 /11. All the experiments and protocols were conducted in strict agreement with the guidelines and ethical principles provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Test Chemical: Crocodile (trade name) was purchased from a local pesticide shop. It contains Imidacloprid- 17.8% SL [1-6-chloro-3-pyridyl methyl-N-nitroimidazolidin-2-ylidene-amine] manufactured by Pioneer Pesticide Pvt. Ltd. Samba district, J & K, India.

Preparation of Stock Solution: According to the experimental design, stock solution was prepared by dissolving crocodile in distilled water as 1 ml solution contained 0.1 ml crocodile.

Collection and Preparation of Aqueous Leaf Extract: The leaves of *Moringa oleifera* were collected from Bundelkhand region. The plant of *Moringa oleifera* was authenticated by RARI-JHS, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Govt. of India (Authentication Reference No.– F.3-27/2006-07/RARI-JHS./Drug Supply/135). The collected leaves were cleaned up with tap water and air-dried in shaded area. The dried leaves were grinded into fine powder. 25 g of powder was mixed with 250 ml hot (98 ºC) distilled water, stayed for 24 h, then filtered with filter paper [25].

Determination of Biochemical Parameters:

Experimental Design: Acclimated fishes were randomly selected and distributed four groups as Group I (4 fishes), Group II (4 fishes), Group III (8 fishes), and Group IV (4 fishes).

Group I: Fishes served as normal control
Group II: Fishes received MLE (12 ml/l)
Group III: Fishes treated with 24 h LC_{50} of IMC (0.423 ml/l)
Group IV: Fishes treated with IMC (0.423 ml/l) + MLE (12 ml/l)

Liver Sampling: After 24 h of the experiment, the fishes were anesthetized, and the liver was dissected out. The liver samples were washed with distilled water and blotted with blotting paper. Liver of 4 fishes from each group Ist, IInd, III^{rd,} and IVth was homogenized in 1 ml ice-chilled physio-logical saline in a glass homogenizer separately. The homogenate was centrifuged at 4000 rpm for 15 min to obtain a clear supernatant. The supernatant was used to analyze Aspartate transaminase, Alanine transaminase, Alkaline Phosphatase, Lactate dehydrogenase, Gamma-Glutamyl transpeptidase and total protein contents.

The same protocol was employed by taking 96 hrs LC_{50} concentration of IMC (0.270 ml/l) and effective concentration of MLE (10 ml/l). A total of three determinations were made for each biochemical parameter. All the parameters were analyzed by biochemical Auto Analyzer using commercially available diagnostic kits.

Statistical Analysis: Results were expressed as mean ± Standard deviation and percent changes (+ and –). The obtained data were analyzed by student t-test to compare means of exposed groups against their normal control groups. The results at $p < 0.05$ were considered as statistically significant.

RESULTS:

Determination of Biochemical Parameters: Results of biochemical parameters induced by Imidacloprid intoxication after 24 h and 96 h and ameliorative effect of *Moringa oleifera* leaf extract on zebra fishes are given in table: 1and 2, **Fig. 1** and **2**.

TABLE 1: EFFECT OF MORINGA LEAF EXTRACT (MLE) ON IMIDACLOPRID (IMC) INDUCED TOXICITY IN ZEBRA FISH, *DANIO RERIO* AFTER 24 h EXPOSURE

	24 h Exposure			
Parameters	**Group- I Control**	**Group- II MLE % COC**	**Group- III IMC % COC**	**Group- IV IMC+ MLE % COC % COT**
AST (IU/L)	65.16 ± 6.85	69.20 ± 2.93 % +6.20	51.56 ± 3.83[a] % -20.87	57.33 ± 2.85 % -12.01 % +11.19
ALT (IU/L)	87.13 ± 7.31	89.10 ± 2.66 % +2.26	95.26 ± 4.09 % +9.33	90.20 ± 2.91 % +3.52

				% -5.31
ALP (IU/L)	413.33 ± 4.28	467.36 ± 4.80 % +13.07	552.63 ± 3.97[a] % + 33.70	497.16 ± 5.15[ab] % +20.28 % -10.03
LDH (IU/L)	644.70 ± 45.16	641.03 ± 7.39 % -0.56	749.40 ± 18.10[a] % +16.24	664.86 ± 11.65[b] % +3.12 % -11.28
GGT (IU/L)	4.53 ± 0.16	4.27 ± 0.02 % -5.73	5.14 ± 0.06[a] % +13.46	4.77 ± 0.09[b] % +5.29 % -7.19
Total Protein (gm/dl)	3.42 ± 0.10	4.07 ± 0.10 % +19.00	2.94 ± 0.08[a] % -14.03	3.32 ± 0.04[b] % -2.92 % +12.92

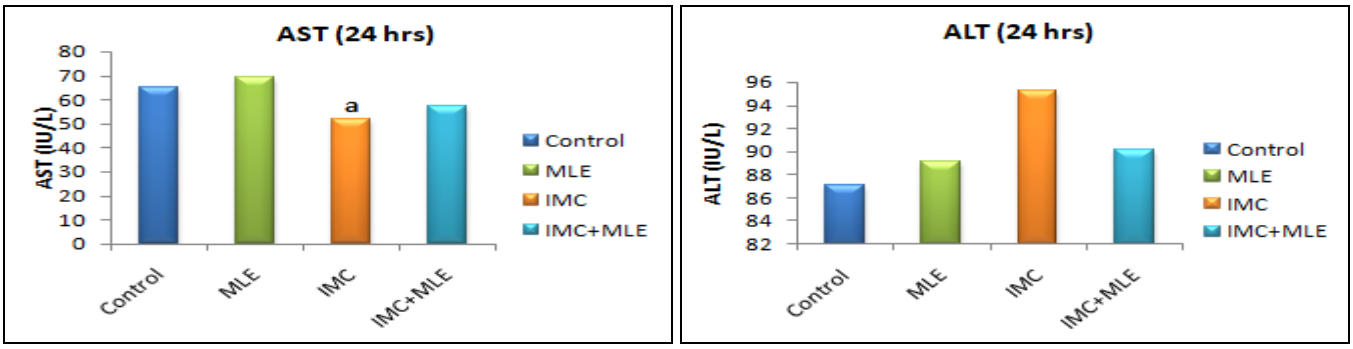
Values are expressed in Mean ± SD, (+, -) denotes increased and decreased, % COC (Change over control), % COT (Change over treated), a: significant when compared to control group (P < 0.05), b: significant when compared to Imidacloprid (IMC) treated group (P < 0.05).

TABLE 2: EFFECT OF MORINGA LEAF EXTRACT (MLE) ON IMIDACLOPRID (IMC) INDUCED TOXICITY IN ZEBRA FISH, *DANIO RERIO* AFTER 96 h EXPOSURE

	24 h Exposure			
Parameters	**Group- I Control**	**Group- II MLE % COC**	**Group- III IMC % COC**	**Group- IV IMC+ MLE % COC % COT**
AST (IU/L)	65.93 ± 4.24	68.90 ± 2.19 % +4.50	74.63 ± 3.46[a] % +13.19	63.86 ± 2.61 % -3.13 % -14.43
ALT (IU/L)	87.96 ± 2.28	91.66 ± 3.12 % +4.20	106.1 ± 5.82[a] % +20.62	84.20 ± 3.80[b] % -4.27 % -20.64
ALP (IU/L)	409.16 ± 9.23	463.3 ± 10.37 % +13.23	546.46 ± 8.00[a] % +33.55	506.23 ± 18.58[ab] % +23.72 % -7.36
LDH (IU/L)	646.36 ± 45.55	642.50 ± 4.92 % -0.59	731.33 ± 34.74 % +13.14	537.86 ± 11.30[b] % -16.78 % -26.45
GGT (IU/L)	4.57 ± 0.04	4.30 ± 0.02 % -5.90	6.10 ± 0.19[a] % +33.47	4.92 ± 0.03[ab] % +7.65 % -19.34
Total Protein (gm/dl)	3.47 ± 0.12	4.06 ± 0.08 % +17.00	3.26 ± 0.06[a] % -6.05	4.13 ± 0.04[ab] % +19.02 % +26.68

Values are expressed in Mean ± SD, (+, -) denotes increased and decreased, % COC (Change over control), % COT (Change over treated), a: significant when compared to control group (P < 0.05), b: significant when compared to Imidacloprid (IMC) treated group (P < 0.05).

The level of ALT and ALP of group III treated with IMC was significantly increased (P< 0.05) than that of control groups in the liver after 24 h and 96 h exposure periods. The activity of AST of treated groups with IMC was decreased after 24 h and increased after 96 h exposure period. The elevated activity of LDH and GGT was also observed in group III over control groups. The lower level in protein content was shown in IMC treated groups at both exposure periods (**Table 1** and **2**, **Fig. 1** and **2**). These tables are also shown that the activity of all the enzymes tends to become normalize after the addition of MLE in group IV.



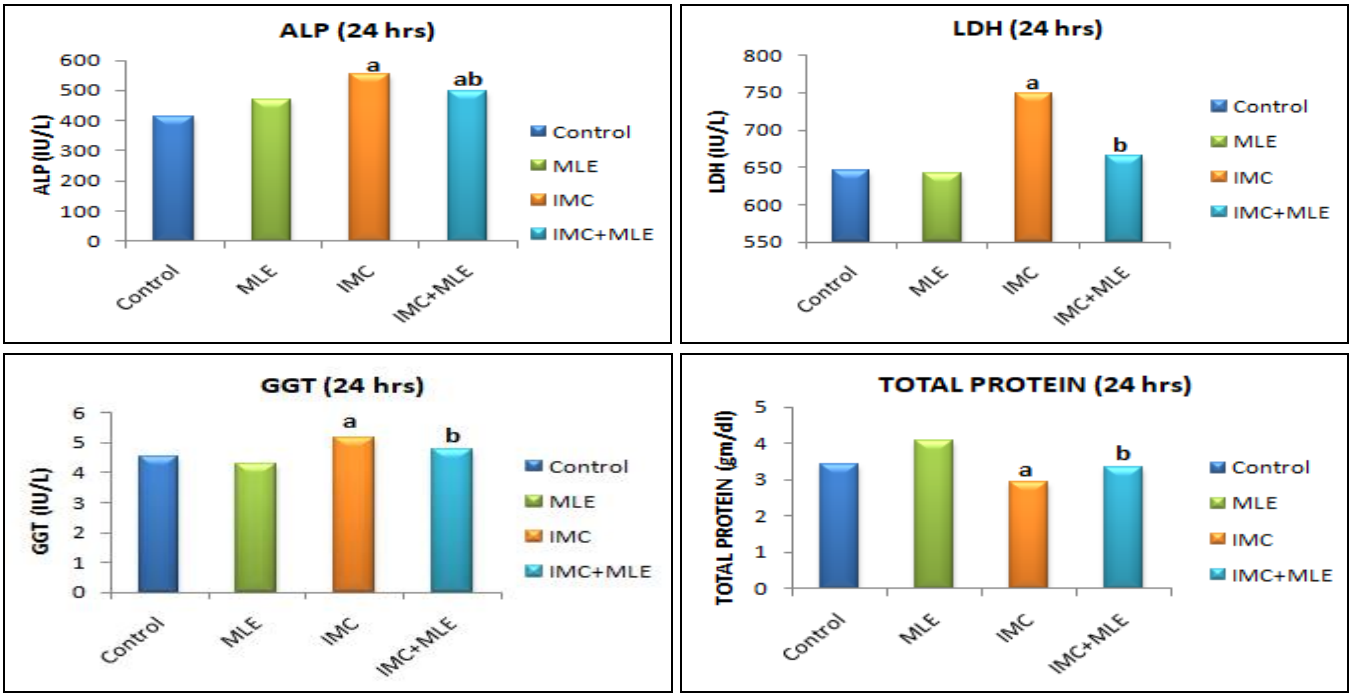


FIG. 1: EFFECT OF MLE ON HEPATIC MARKERS IN IMC INDUCED LIVER TOXICITY IN ZEBRA FISHES AFTER 24 h EXPOSURE PERIOD

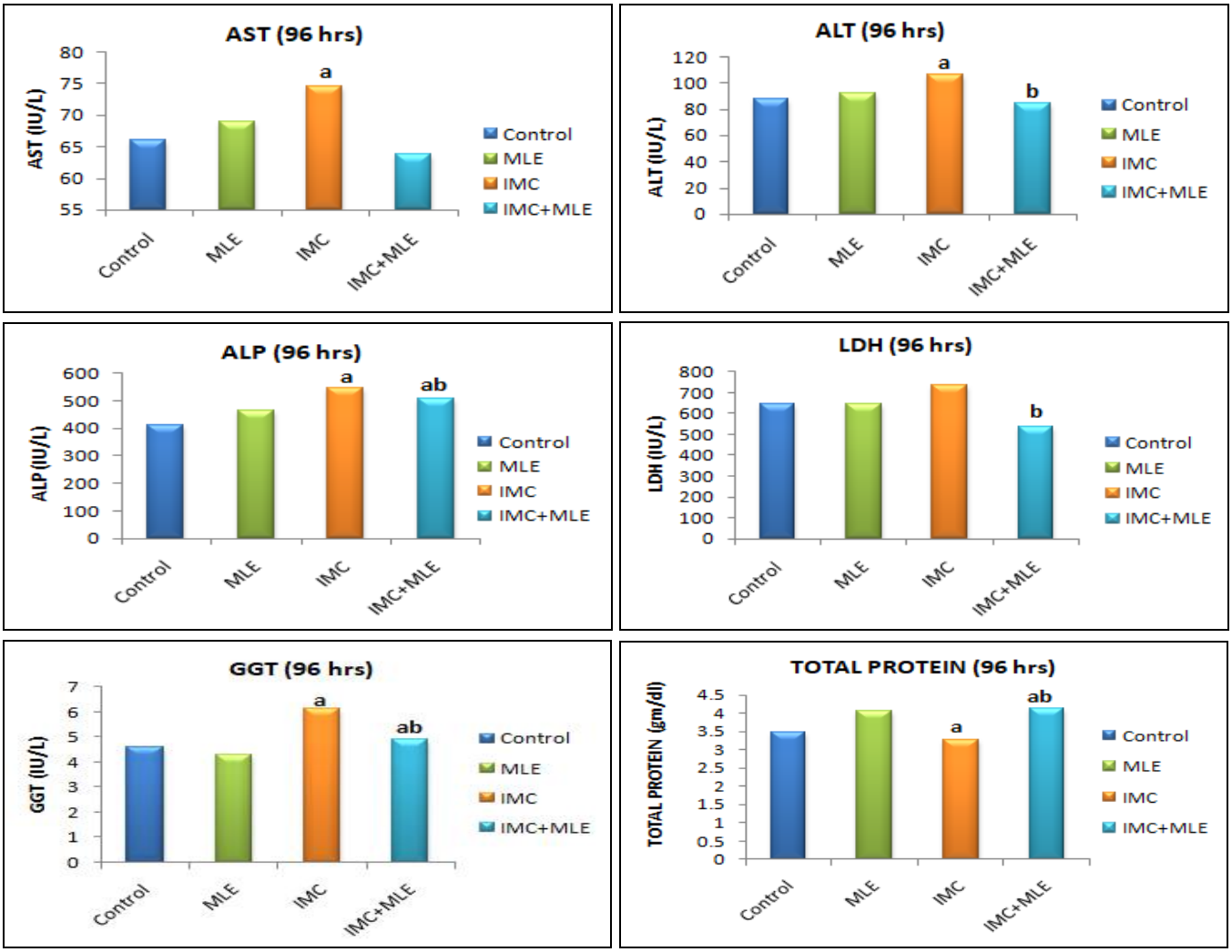


FIG. 2: EFFECT OF MLE ON HEPATIC MARKERS IN IMC INDUCED LIVER TOXICITY IN ZEBRA FISHES AFTER 96 h EXPOSURE PERIOD

DISCUSSION: The liver is a versatile metabolic center where most xenobiotics are metabolized and detoxified. It regulates many biochemical reactions, which can be altered under stressed conditions. In the present study, homogenates of the liver were used for enzyme analysis to assess the proper functioning of the liver. AST activity was decreased after 24 h and increased after 96 hrs exposure period of imidacloprid (IMC) in zebra fishes. It has been suggested that decreased level may counteract the toxic effects, maintaining the integrity of hepatocytes, the structure of the membrane, and balance the body against toxic substances in fishes [9]. On increasing the exposure period increased level of AST was observed, which indicates that the cells are completely destroyed, and the enzyme is released due to the loss of functional integrity of cell membrane [29].

The level of ALT was increased significantly throughout the experimental periods. The elevation may be due to disturbances in Kreb's cycle and hepatic damage caused by IMC. The elevated levels are in agreement with earlier reports of fishes exposed to toxicants [8, 30]. ALP and LDH are important liver enzymes that serve as biomarkers for the evaluation of cellular responses to hepatotoxicity. ALP plays a significant role in phosphate hydrolysis to provide energy and transport of metabolites across the cell membrane.

LDH catalyzes the conversion of pyruvic acid to lactic acid, thus switching on the anaerobic respiration from the aerobic respiration. **Tables 1 and 2** indicate the increased level of ALP and LDH following 24 h and 96 h exposure. The elevated levels of ALP may indicate an increase in the rate of phosphorylation and an accelerated membrane transport function which was supported by several workers in fishes [30, 31]. The high LDH activity also suggested that the stressed animals were meeting high energy demand through anaerobic oxidation [32]. GGT plays a key role in the gamma-glutamyl cycle, which is a pathway for synthesis and degradation of glutathione and xenobiotic-detoxification [33]. Evidence suggests that GGT also exerts a pro-oxidant role with regulatory effects in cellular pathophysiology [34, 35]. In the present study, GGT level increased during all exposure periods showing the oxidative damage caused by Imidacloprid. These aforesaid alterations of liver enzymes in zebra fishes are supported by preceding studies [3, 4, 36].

The current study showed the reduced level of total protein after 24 h and 96 h exposure of IMC in zebra fishes. Depletion in protein may be due to their degradation, possible utilization of degraded products for metabolic processes, and stress-mediated immobilization to obtain more energy. The protein depletion may be due to loss of protein either by reducing protein synthesis or increased proteolytic activities [37, 38]. It could be attributed to the impaired assimilation of amino acids in the polypeptide chain in protein synthesis.

Prasad *et al.* (2002) [39] showed that total protein level decreased whereas free amino acid levels increased after cypermethrin exposure in *Labeo rohita*. The increased free amino acid level suggests tissue damage due to increased proteolytic activities under the stressed condition, and these amino acids can be utilized for energy production through transamination. Necrosis of liver cells may be another reason to elevate the enzyme activities during intoxication [40, 41]. Lipid peroxidation is a free radical-mediated chain of reactions when initiated, results in an oxidative deterioration of polyunsaturated lipids. Earlier researches exhibited that the ROS is produced after Imidacloprid intoxication in zebra fishes [14].

Moringa oleifera leaf extract (MLE) possesses anticancer, anti-inflammatory, bacteriocidal, hypocholesterolemic, antioxidant, neuro, and hepatoprotective properties [42, 43]. Ameliorative effects of *Moringa oleifera* have been studied against many chemicals by several researchers [5, 40, 44]. Administration of MLE restored the activities of AST, ALT, ALP, LDH, GGT, and total protein toward the normal level, as shown in our study. An elevated level of GGT being a pro-oxidant, as observed in this investigation, shows the inhibition of antioxidant system, but on the addition of MLE it was decreased, indicating the antioxidant property of *Moringa* leaves. The normalization is an indication of hepatoprotective effect against Imidacloprid. The reversal of increased trans-aminases by MLE supplement may be due to the healing of hepatic parenchyma and regeneration of hepatocytes. Moringa may have induced the repairing effect on the damaged liver by its

essential amino acid, such as methionine and cysteine, which boost the total protein contents, as evidenced by several authors [34, 45].

CONCLUSION: The present study revealed that MLE possesses hepatoprotective potential due to antioxidant properties. MLE normalizes the altered values of hepatic enzymes in IMC intoxicated zebra fishes, which justifies that *Moringa oleifera* leaf can be used in the treatment of various hepatic diseases and disorders by boosting the oxygen-free radical scavenging system.

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REFERENCES:

1. Qadir S, Latif A, Ali M and Iqbal F: Effects of Imidacloprid on hematological and serum biochemical profile of *Labeo rohita*. Pakistan Journal of Zoology 2014; 46(4): 1085-90.
2. Tyor AK and Harkrishan: Effects of Imidacloprid on viability and hatchability of embryos of the common carp (*Cyprinus carpio* L.). International Journal of fisheries of Aquatic Studies 2016; 4(4): 385-89.
3. Alian SH, Shahsavani D and Baghyshni H: Study of tissue enzymes change of cyanide poisoning in common carp. Iranian Veterinary Journal 2013; 8(4): 44-51.
4. Begum G: *In-vivo* biochemical changes in liver and gill of Clarias batrachus during cypermethrin exposure and following cessation of exposure. Pesticide Biochemistry Physiology 2005; 82: 185-96.
5. Christian EO, Samuel CM, Chundi ED, Anaelechi JO, Chinwemma FO, Daniel LA, John KN and Obi E: Amelioratory effect of methanolic leaf extract of *Moringa oleifera* on some liver and kidney function and oxidative stress markers in lead intoxicated rats. European Journal of Medicinal Plants 2016; 12(4): 1-12.
6. Priya BP and Maruthi YA: Imidacloprid toxicity on biochemical constituents in liver tissue of fresh water teleost *Channa punctatus*. International Journal of Pharma and Bio Sciences 2013; 4(4): (B) 50-55.
7. Wast N, Prakash MM, Gupta AK and Gaherwal S: Acute toxicity assessment of Imidacloprid 70% WG to a Guppy, *Poecilia reticulata* (Peters). Global Veterinaria 2016; 17(3): 245-49.
8. Gabriel UU, Akinrotimi OA and Ariweriokuma VS: Changes in metabolic enzymes activities in selected organs and tissue of *Clarias gariepinus* exposed to cypermethrin. Chemical Engineering Journal 2012; 1(1): 25-30.
9. Lohner TW, Reash RJ and Williams M: Assessment of tolerant sunfish populations (Lepomis spp.) inhabiting selenium- laden coal ash effluent. 2. Tissue biochemistry evaluation. Ecotoxicology and Environmental Safety 2001; 50: 217-24.
10. El-Gendy KS, Aly NM, Mahmoud FH, Kenawy A and El-Sebae AK: The role of vitamin C as antioxidant in protection of oxidative stress induced by imidacloprid. Food and Chemical Toxicology 2010; 48: 215-21.
11. Chen XY, Shao JZ, Xiang LX and Liu XM: Involvement of apoptosis in malathion-induced cytotoxicity in a grass carp (*Ctenopharyngodon idellus*) cell line. Comparative Biochemistry and Physiology- Part C: Toxicology & Pharmacology 2006; 142(1): 36-45.
12. Das BK and Mukharjee SC: Toxicity of cypermethrin in Labeo rohita fingerlings: Biochemical enzymatic and hematological consequences. Comparative Biochemistry and Physiology Part- C: Toxicology & Pharmacology 2003; 134: 109-21.
13. Uner N, Oruc EO, Canli M and Sevgler Y: Effects of cypermethrin on antioxidant enzyme activities and lipid peroxidation in liver and kidney of the freshwater fish, *Oreochromis niloticus* and *Cyprinus carpio* (L.). Bulletin of Environmental Contamination and Toxicology 2001; 67: 657-64.
14. Ge W, Yan S, Wang J, Zhu L, Chen A and Wang J: Oxidative stress and DNA damage induced by imidacloprid in Zebra fish (*Danio rerio*). Journal of Agricultural and Food Chemistry 2015; 63(6): 1856-62.
15. Saalu LC, Ogunlade B, Ajayi GO, Oyewopo AO, Akunna GG and Ogunmodede OS: The hepato-protective potentials of *Moringa oleifera* leaf extract on alcohol-induced hepato-toxicity in Wistar rat. American Journal of Biotechnology and Molecular Sciences 2012; 2(1): 6-14.
16. Vyas S, Kachhwaha S and Kothari SL: Comparative analysis of phenolic contents and total antioxidant capacity of *Moringa oleifera* Lam. Pharmacognosy Journal 2015; 7(1): 44-51.
17. Fitriana WD, Ersam T, Shimizu K and Fatmawati S: Antioxidant activity of *Moringa oleifera* extract. Indonesian Journal of Chemistry 2016; 16(3): 297-301.
18. Karthivashan G, Tangestani FM, Arulselvan P, Abas F and Fakurazi S: Identification of bioactive candidate compounds responsible for oxidative challenge from hydro-ethanolic extract of *Moringa oleifera* leaves. Journal of Food Science 2013; 78: C1368-1375.
19. Torres-Castillo JA, Sinagawa-Garcia SR, Martenez-Avila GCG, Lopez-Flores AB, Sanchez-Gonzalez EI, Aguirre-Arzola VE, Torres-Acosta RI, Olivares-Saenz E, Osorio-Hernandez E and Guteirrez-Diez A: *Moringa oleifera*: phytochemical detection, antioxidants, enzymes and antifungal properties. International Journal of Experimental Botany 2013; 82: 193-202.
20. Jimenez- Arellanes A, Meckes M, Ramireez R, Torres J and Luna-Herrera J: Activity against multidrug- resistant *Mycobacterium tuberculosis* in Mexican plants used to treat respiratory disease. Phytotherapy Research 2003; 17: 903-08.
21. Patel JP, Gami B and Patel K: Evaluation of in vitro Schizonticidal properties of acetone extract of some Indian medicinal plants. Advances in Biological Research 2010; 4(5): 253-58.
22. Sreelatha S and Padma PR: Antioxidant activity and Total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Food for Human Nutrition 2009; 64: 303-11.
23. Al Otaibi LS: Role of Moringa olifera leaves extract against Cadmium in Mice. World Journal of Research and Review 2016; 3(6): 38-44.

24. Sheikh A, Yeasmin F, Agarwal S, Rahman M, Islam K, Hossain S, Karim Md R, Nikkon F, Saud ZA and Hossain K: Protective effects of *Moringa oleifera* Lam. leaves against arsenic induced toxicity in mice. Asian Pacific Journal of Tropical Biomedicine 2014; 4(suppl 1): S353-S358.
25. Khalil F and Korni FMM: Evaluation of Moringa oleifera leaves and their aqueous extract in improving growth, immunity and mitigating effect of stress on common carp (*Cyprinus carpio*) fingerlings. Turkish Journal of Aquatic Sciences 2017; 32(3): 170-77.
26. Achuba FI, Ubogu LA and Ekute BO: Moringa oleifera attenuates crude oil contaminated diet induced biochemical effects in Wistar Albino Rats. UK Journal of Pharmaceutical Biosciences 2016; 4(5): 70-77.
27. Khalil RH, Diab AM, Shakweer MS, Ghetas HA and Khallaf M: Amelioratory effect of methanolic leaf extract of *Moringa oleifera* on some liver function and oxidative stress markers in sodium fluoride toxicity in sea bream *Sparus auratus* L. European Journal of Pharmaceutical and Medical Research 2018; 5(2): 11-22.
28. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, Collins JE, Humphray S, McLaren K and Matthews L: The zebra fish reference genome sequence and its relationship to the human genome. Nature 2013; 496(7446): 498-503.
29. Kalender S, Uzun FG, Durak D, Demir F and Kalender Y: Ion-induced hepatotoxicity in rats: The effects of vitamins C and E. Food and Chemical Toxicol 2010; 48: 633-63.
30. Selamoglu Talas Z, Pinar Dundar S, Fuat Gulhan M, Orun I and Kakoolaki, S: Effects of propolis on some blood parameters and enzymes in carp exposed to arsenic. Iranian Journal of Fisheries Sciences 2012; 11(2): 405-14.
31. Yaji AG, Auja J, Oniye SJ, Adakole JA and Usman JI: Effect of cypermethrin on behavior and biochemical indices of fresh water fish, *Oreochromis niloticus*. Electronic Journal of Environmental, Agricultural and Food Chemistry 2011; 10: 1927-34.
32. Doss PJ, Sreelatha P, Sulthana RN and Lakshmi Bai VK: Effect of cypermethrin on selective biochemical parameters in a fish *Heteropneustes fossilis*. Aquaculture 2008; 9: 205-10.
33. Courtay C, Oster T, Michelet F, Visvikis A, Diederich M, Wellman M and Siest G: "Gamma- glutamyltransferase: nucleotide sequence of the human pancreatic cDNA. Evidence for a ubiquitous gamma-glutamyltransferase polypeptide in human tissues". Biochemical Pharmacology 1992; 43(12): 2527-33.
34. Dominici S, Paolicchi A, Corti A, Maellaro E and Pompella A: Prooxidant reactions promoted by soluble and cell-bound gamma-glutamyltransferase activity. Methods in Enzymology 2005; 401: 484-501.
35. Lee DH and Jacobs DR Jr: Is serum gamma-glutamyltransferase a marker of exposure to various environmental pollutants? Free Radical Research 2009; 43(6): 533-7.
36. Nanda NBP, Das PC, Jena JK and Sahoo PK: Changes in selected haematological and enzymatic parameters of *Heteropneustes fossilis* exposed to sub-lethal toxicity of rotenone. Indian Journal of Fisheries 2010; 57(1): 73-80.
37. Bhanu AP and Deepak M: Impact of Cypermethrin on biochemical aspects of clinical importance in the blood of fresh water fish *Cyprinus carpio*. Journal of Entomology and Zoology Studies 2015; 3(1): 126-28.
38. Malla FA: Assesment of total protein concentration in liver of fresh water fish, *Channa punctatus* (Bloch) with special reference to an organophosphte insecticide, chlorpyrifos. International Journal of Pharma and Bio Sciences 2012; 3(2): 567-71.
39. Prasad D and Veeraiah K: Effect of Cypermethrin on protein metabolism of the fish, Labeo rohita (Hamilton). Bulletin of Pure and Applied Sci 2002; 21A(1): 27-32.
40. Jyotsna and Swarnalatha Y: Effect of flavonoids in Acetaminophen Induced Liver Injury in *Danio rerio*. International Journal of Health Sciences and Research 2016; 6(2): 352-59.
41. Kim JH, Ee SM, Jittiwat J, Ong ES, Farooqui AA and Jenner AM: Increased expression of acyl-coenzyme A: Cholesterol acyltransferase-I and elevated cholesteryl esters in the hippocampus after excitotoxic injury. Neuroscience 2011; 185: 125-34.
42. Ghasi S, Nwobobo E and Ofili JO: Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed Wistar rats. Journal of Ethnopharmacology 2000; 69: 21-25.
43. Ujah OF, Ujah IR, Johnson JT and Oka VO: Effect of ethanolic leaf extract of *Moringa oleifera* leaf on haematological and biochemical parameters of Wistar rats. Journal of Natural Product and Plant Resources 2013; 3(2): 10-14.
44. Pari L and Kumar NA: Hepatoprotective activity of *Moringa oleifera* an antitubucular drug-induced liver damage in rats. J of Medicinal Food 2002; 5(3): 171-77.
45. Ekam VS, Johnson JT, Dasofunjo K, Odey MO and Anyahara SE: Annals of Biological Research 2012; 3(12): 5590-94.

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