Life Science Bulletin, Vol. 19(1&2) 2022 : 01-04 ISSN : 0973-5453 (Print), 2321-7952 (Online)

THE EFFECT OF LESENTA INSECTICIDE ON MALONDIALDEHYDE (MDA) LEVELS IN DIFFERENT TISSUES OF COMMON CARP, *CYPRINUS CARPIO* L.

MUMMADI NARSING RAO*, PALLAVI CHAVAN and R. P. MALI

S.G.B. Mahavidyalaya, Purna dist. Parbhani (M.S).
Principal, Indira Gandhi (Sr) College, Cidco, Nanded (M.S.)

ABSTRACT : The effect of insecticide Lesenta (40% Imidacloprid+40% Fipronil) bioaccumulation in liver, brain and gills of common carp (*Cyprinus carpio*) was investigated on Malaondialdehyde levels. In this study similar sized *Cyprinus carpio* fishes were selected and divided into three groups : control group-I and group-II, group-III were exposed to different sub-lethal concentrations 1/10 (1 mg/l) LC_{50} and 1/5(2 mg/l) LC_{50} of Lesenta respectively for 28th days. The fish liver, brain and gills were removed, cleaned and weighed. Prepared a clear supernatant and used for estimation of MDA levels in tissues. Final results showed that Lesenta insecticide effected the lipid peroxidation, MDA levels have increased in all tissues. The current findings suggest that oxidative stress is a factor in lesenta induced hepatic, brain and gill toxicity. As a result of this exposure oxidative stress may occur, resulting in reduced cellular function which can lead to diseases or death of fish.

Key words : *Lesenta, Insecticide, Cyprinus carpio, Lipid peroxidation, Malondialdehyde.*

INTRODUCTION

In humans, the antioxidant system works by limiting the harmful effects of excessive reactive oxygen system (ROS) production during stressful situations, allowing for improved cellular function of various organelles, which is important for reproductive success. They are accessible in both enzymatic and non-enzymatic forms (Mahvash *et al.*,2014). This antioxidant defense system works in three ways : prevention with antioxidants, interception with antioxidants, and repair with antioxidants. In steroidogenic tissues, mitochondria and metabolic activities are critical sites for the formation of physiological reactive oxygen species (ROS) (Agnes *et al.*, 2015). The mechanisms that cause stress-induced tissue injury are still unknown. Although evidence suggests that excessive production of free radicals is a key factor in these processes.

The antioxidant system differs from one tissue to the next and from one cell type to the next. SOD, CAT, GPx and GST are the most significant *in vivo* enzymatic antioxidant defense systems. Ceruloplasmin, transferrin, glutathione (GSH), albumin, ferritin, myoglobin, and metallothionein are non-enzymatic antioxidants. They may act as antioxidants by inactivating pro-oxidants and scavenging free radical activity (Agarwal *et al.*,2008 and Fujii *et al.*,2005). Dietary antioxidants, endogenous enzymatic and non-enzymatic elements that regulate the overproduction of these ROS are all examples of antioxidant defense systems (Lu *et al.*,2003). Malondialdehyde (MDA) is one of the most extensively utilized lipid peroxidase (end products of unsaturated fats) markers in evaluating oxidative stress measurement in experimental animals (Stanton *et al.*,1992 and Anisman *et al.*,2000). Catalase (CAT) and Superoxide Dismutase (SOD) activities of various tissues are the most important *in vivo* enzymatic antioxidants of attention in experimental study because they are important ROS neutralizing antioxidants and indexes of peroxidation.

Fishes, among the different creatures that make up the aquatic ecosystem, are the ones that are most sensitive to changes in their surroundings. The concentration of pesticides in aquatic species appears to be several times higher than that found in the ecosystem. This is due to bioaccumulation, which occurs when harmful compounds are taken in by the organism from the environment and deposited in numerous organs and tissues. Because of biomagnification, toxic chemicals become increasingly concentrated at higher trophic levels. Pesticides released into aquatic ecosystems have a significant impact on fish, and thus on humans.

The presence of toxicants in aquatic creatures exposed to pesticides is linked to the generation of reactive oxygen species (ROS), which produces oxidative stress, a probable mechanism of toxicity (Oropesa *et al.*,2008). Fish have superoxide dismutase, catalase, peroxidase, and glutathione S-transferase as defense mechanisms against free radicals (Guven *et al.*,2008).

The major enzyme responsible for converting O-2 to H_2O_2 is superoxide dismutase. Catalase then converts hydrogen peroxide into oxygen and water. Peroxidase is also a peroxidase enzyme that transforms a group of peroxides, including hydrogen peroxide (Hermes,2004 and Maran *et al.*, 2009). Glutathione-S-transferase is engaged in the biotransformation of harmful chemicals into less toxic compounds that fish may easily excrete. The current study examined the oxidative stress parameters of *C.carpio* liver, brain, and gills exposed to different sub-lethal concentrations (1/10th LC_{50} and 1/5th LC_{50}) of Lesenta insecticide.

*Corresponding author (email: mummadinarsingrao73@gmail.com)

Received 12.03.2022 Accepted 25.06.2022

MATERIAL AND METHODS

Test insecticide : For the toxicity investigation on physiological changes in *Cyprinus carpio*, the test chemical, Lesenta (Imidacloprid 40%+Fipronil 40%), was bought from a local market. This study used 1/10th and 1/5th of the sub-lethal concentrations of lesenta (*i.e.*, 1 mg/l and 2 mg/l, respectively) based on the toxicity study. In distilled water, the various concentrations were made.

The experimental design was as follows : Following acclimation, healthy and similar sized *Cyprinus carpio* (13.18± 0.38 gm and 9.09±0.29 cm in length) were selected and divided into three groups of ten fish each.

Group I - Control fishes

Group II - For 28th days, fish were exposed to 1/10th of the LC_{50} value of Lesenta (1 mg/l).

Group III - Fish exposed for 28th days to 1/5th of the LC_{50} value of Lesenta (2 mg/l).

Fish were treated to their respective sub-lethal concentrations of Lesenta and kept at these levels for the duration of the experiment. The test medium was changed on a daily basis, allowing for the elimination of nitrogenous waste emitted by the test fishes as well as unconsumed food. Fish were dissected after the control and experimental groups were completed to measure antioxidant enzymes in the liver, gills, and brain tissues.

Tissue preparation : The liver, brain, and gills were removed, cleaned, and weighed from the sacrificed animals. In cold saline, a ten percent (w/v) homogenate of various tissues was generated using a homogenizer fitted with a Teflon pestle. In a chilled centrifuge, the homogenate was first centrifuged at 2000 rpm for 15 minutes. The pellet, which contains a nuclear fraction and cell detritus, is thrown away. For the post mitochondrial supernatant, spin the supernatant at 8000 rpm for 20 minutes. This clear supernatant was then used for crude enzyme research.

Estimation of MDA (malondialdehyde) : The approach described by Nadigar was used to estimate MDA in tissue (1986).

Principle : Malonaldehyde (MDA), a byproduct of unsaturated fatty acid peroxidation, can combine with thiobarbituric acid (TBA) to generate thiobarbituric acid-reactive material, a colorful complex (TBARS). MDA and thiobarbituric acid create a 1:2 adduct that may be detected by spectrophotometry at 531 nm.

Reagents :

- 40% TCA : 40 grams of TCA were dissolved in distilled water and diluted to 100 ml.
- TCA concentration of 5% : 50 g TCA was dissolved in distilled water and diluted to 1000 ml. This was kept in the refrigerator.
- 6.7 g TBA was dissolved in distilled water and formed up to 1000 ml of 0.67% Thiobarbituric acid.

Procedure : In a test tube, 1 ml homogenate/serum was combined with 1 ml 40% TCA, followed by 2 ml TBA. For 20 minutes, the tube was immersed in boiling water. After cooling, the supernatant was centrifuged and the absorbance of the supernatant against distilled water was measured at 535 nm.

Table. 1 The effect of lesenta on the malondialdehyde (MDA) levels from the liver of fish, *C.carpio*.

Duration (days)	MDA levels (nmol/gr) in liver tissue of control and experimental fishes				
	Control (Group I)	1/10th of LC_{50} value of Lesenta (1 mg/l) (Group II)	Percent of variations	1/5th of LC_{50} value of Lesenta (2 mg/l) (Group III)	Percent of variations
7	0.19±0.04	0.21±0.07	10.53%	0.22±0.01	15.79%
14	0.20±0.01	0.23±0.01	15.00%	0.25±0.02	25.00%
21	0.18±0.03	0.20±0.03	11.11%	0.26±0.01	44.44%
28	0.19±0.04	0.24±0.02	26.32%	0.28±0.05	47.37%

Each Datum represents Mean ±SEM of ten individuals (n=10).

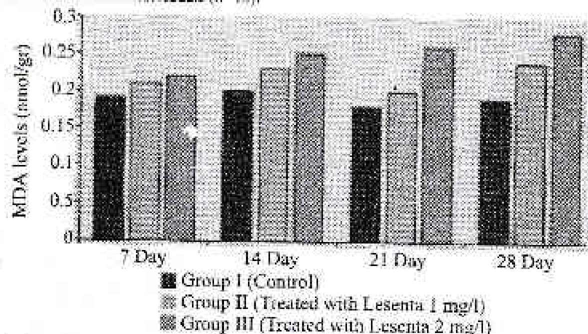

Fig. 1 The effect of lesenta on MDA levels (nmol/gr) from the liver of fish, *C.carpio* ±SE (n=10).

Co-ordinator
IQAC
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn) Dist. Parbhani - 431511 (M.S.)

PRINCIPAL
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn.) Dist.Parbhani

RESULTS AND DISCUSSION

a) In Liver : Figure & Table indicate changes in MDA levels in the liver, brain, and gills of carp subjected to 1 mg/l & 2 mg/l Lesenta. MDA is a key biomarker of oxidative damage, and MDA levels in the liver, brain, and gill tissues of Lesenta-treated animals were modestly different from the control group ($p<0.005$). Significant increases in MDA levels of liver, brain, and gill tissues were identified in fish exposed to lesenta when compared to the control group, while MDA levels of tissues were found to be lower in the Lesenta treated group when compared to the control group (Table.1 & Fig.1).

b) In Brain : MDA levels in the brains of lesenta treated fishes are high, as seen in the figure & table. When compared to control fishes, MDA levels in group II fishes (1 mg/l treated) increased from 67%-87% on the 7th and 28th day of lesenta exposure. MDA levels in the brain of 2 mg/l lesenta treated fishes are 2.01, 2.09, 2.5 & 2.6 nmol/gr after 7th days, 14th days, 21st days and 28th days of exposure, respectively, and rose by 76%, 83%, 98% and 103% when compared to control fishes (Table.2 & Fig.2). At $p<0.001$, the increasing amounts were statistically significant.

c) In Gills : After 7th, 14th, 21st and 28th days of exposure, the MDA mean values in control group fish are 1.69±0.18, 1.61±0.03, 1.68±0.04 and 1.62±0.04. MDA levels in group II fishes increased from 2.01 nmol/gr (26%) to 2.64 nmol/gr (62%) following the 7th and 28th exposures, and these changes are statistically significant at p 0.001. MDA levels for the 7th, 14th, 21st, and 28th days of exposure in group III fishes (2 mg/l treated) are 2.12±0.06, 2.51±0.04, 2.66±0.08 & 2.88±0.01 nmol/gr, respectively, and these values are increasing 33%, 55%, 58% & 77% when compared to control group fishes (Table.3 & Fig.3).

Lipid peroxidation, which is caused by the oxidative destruction of saturated and unsaturated lipids, has long been employed as a measure of oxidative damage in fish subjected to heavy metal-induced environmental stress (Gioda *et al.*, 2007). MDA levels increased after exposure to arsenic in all of the tissues tested in our investigation. These findings strongly suggest that arsenic caused the fish to experience widespread oxidative stress. As a result, increased ROS production could be attributed to arsenic poisoning, while additional consequences including enzymatic inhibition or genotoxic damage could also occur.

Free radical damage and oxidative stress in diverse fish exposed to hazardous metals and organic pollutants (e.g., cadmium, mercury, copper, arochlor, and contaminated sediments) have been examined in recent ecotoxicological research (Talas and Gulhan,2009). MDA levels in the liver, gills, and muscle tissues increased as a result of extensive tissue lipid peroxidation (Berntssen *et al.*,2003).

Some studies have found increased MDA levels in aquatic organisms exposed to heavy metals (Pandey *et al.*, 2008). MDA levels in the liver, gills, and muscle of fish exposed to arsenic increased in our study as a sign of oxidative stress, but these increases were substantially less pronounced in the arsenic+propolis group's tissues. The administration of propolis in this circumstance lowered oxidative stress. These

Table. 2 The Effect of lesenta on the malondialdehyde (MDA) levels from the brain of fish, *C.carpio*.

	MDA levels (nmol/gr) in brain tissue of control and experimental fishes				
Duration (days)	Control (Group I)	1/10th of LC_{50} value of Lesenta (1 mg/l) (Group II)	Percent of variations	1/5th of LC_{50} value of Lesenta (2 mg/l) (Group III)	Percent of variations
7	1.14±0.14	1.91±0.07	67.54%	2.01±0.08	76.32%
14	1.14±0.06	2.01±0.03	76.32%	2.09±0.05	83.33%
21	1.26±0.07	2.30±0.04	82.54%	2.5±0.02	98.41%
28	1.28±0.09	2.40±0.08	87.50%	2.6±0.04	103.13%

Each Datum represents Mean ±SEM of ten individuals (n=10).

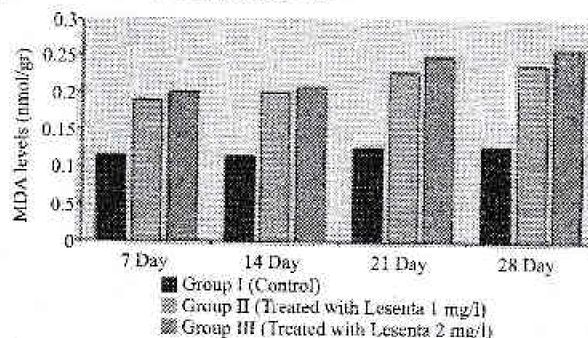

Fig. 2 The effect of lesenta on MDA levels (nmol/gr) from the brain of fish. *C.carpio* ±SE (n=10).

Co-ordinator
IQAC
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn) Dist. Parbhani - 431511 (M.S.)

PRINCIPAL
Shri Guru Buddhiswami Maha...
Purna (Jn.) Dist...

Table. 3 The Effect of lesenta on the malondialdehyde (MDA) levels from the gill of fish, *C.carpio*.

Duration (days)	MDA levels (nmol/gr) in gill tissue of control and experimental fishes				
	Control (Group I)	1/10th of LC_{50} value of Lesenta (1 mg/l) (Group II)	Percent of variations	1/5th of LC_{50} value of Lesenta (2 mg/l) (Group III)	Percent of variations
7	1.69±0.18	2.01±0.02	26.42%	2.12±0.06	33.33%
14	1.61±0.03	2.36±0.02	46.58%	2.51±0.04	55.90%
21	1.68±0.04	2.56±0.07	52.38%	2.66±0.08	58.33%
28	1.62±0.04	2.64±0.06	62.96%	2.88±0.01	77.78%

Each Datum represents Mean ±SEM of ten individuals (n=10).

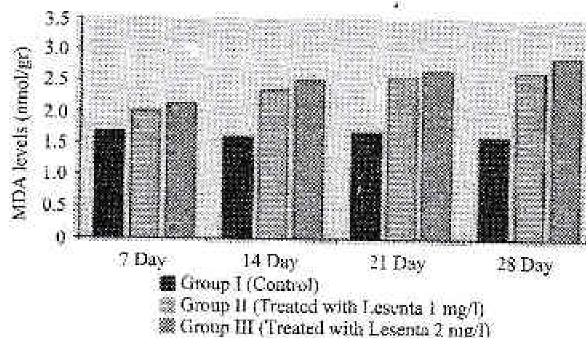

Fig. 3 The effect of lesenta on MDA levels (nmol/gr) from the gill of fish, *C.carpio* ±SE (n=10).

findings give direct evidence for propolis' protective role in the antioxidative defense system against heavy metals, which provides therapeutic qualities on the antioxidative defense system. A propolis concentration of 10 mg l1 may contribute to the antioxidative defense system of carp, according to biochemical studies (Ramanathan *et al.*,2020).

REFERENCES

Agarwal, R. (2008). *Am. J. Emerg. Med.*, **25** : 844-856.

Agnes, S.; Ward, C. and Jose, P. E. (2015). *International Journal of Stress Management*. pp. 1-19

Berntssen, M. H.; Aatland, A. and Handy, R. D. (2003). *Aquat. Toxicol.*, **65** : 55-72.

Fujii, J.; Iuchi, Y. and Okada, F. (2005). *Biol. Endocrinol.*

Gioda, C. R.; Lissner, L. A.; Pretto, A. and da Rocha, J. B. (2007). *Chemosphere*, **69** : 170-175.

Guven, A.; Gul, S.; Kaya, I.; Nur, G.; Deveci, A. and Ka; a, O. (2008). *Kafkas Univ. Vet. Fakul. Tesi Derg.*, **14** : 13-18.

Hermes, L. M. (2004). Oxygen in biology and biochemistry. In : *Functional metabolism : Regulation and adaptation* (ed. K. B. Storey). John Wiley & Sons Inc., Hoboken, USA, pp. 319-368.

Lu, L. G.; Zeng, M. D. and Mao, Y. M. (2003). *World J. Gastroenterol.*, **12** : 2796-2800.

Mahvash. J.; Maryam, S.; Homeira, Z. and Fatemeh, R. (2014). *EXCLI Journal*, **13** : 161-171

Maran, E.; Fernandez, M.; Barbieri, P.; Font, G. and Ruiz, M. J. (2009). *Ecotoxicol. Environ. Safe*, **72** : 922-930.

Oropesa, A. L.; Cambero, J. P. G. and Soler, F. (2008). *Environ. Toxicol.*, **23** : 285-293.

Pandey, S.; Parvez, S.; Ansari, R. A. and Ali, M.(2008). *Chem. Biol. Interact*, **174** : 183-192.

Ramanathan, K.; Shila, S.; Kumaran, S. and Panneerselvam C (2020). *Hum. Exp. Toxicol.*, **22** : 129-136.

Stanton, A. L.; Tennen, H.; Affleck, G. and Mendola, R. (1992). *J. Social Clin. Psychol.*, **11** : 1-13.

Talas, Z. S. and Gulhan, M. F. (2009). *Ecotoxicol. Environ. Saf.*, **72** : 1994-1998.

Co-ordinator
IQAC
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn) Dist. Parbhani - 431511 (M.S.)

Estd. 1983

PRINCIPAL
Shri Guru Buddhiswami Mahavidyalaya