Indian J. Applied & Pure Bio. Vol. 38(2), 597-607 (2023). **ISSN: 0970-2091**
A web of Science Journal

Histopathological alterations in liver of walking catfish, *Clarias batrachus* (Linnaeus, 1758) exposed to sub- lethal concentration of hybrid pesticide containing chlorpyrifos (50 %) and cypermethrin (5 % EC)

***Aman Kumar, Deepshikha Samdershi, Uzma Shamim and Priti Kumari**

University Department of Zoology, Ranchi University, Ranchi-834008 (India)

***Corresponding author-** Email id- amankumar007official@gmail.com

Abstract

Recent trend of using hybrid pesticides in agricultural practices have drawn the attention of researchers to investigate their cumulative effects on the non- target organisms, especially fish. In the present study, toxic effects of hybrid pesticide containing chlorpyrifos (50%) and cypermethrin (5% EC) on liver of *Clarias batrachus* have been investigated. Healthy fish of 20-25 cm length and 75-100 g weight were purchased from local fish market in Ranchi and were kept for acclimatization in laboratory conditions for two weeks. The fish were divided into 3 groups (n= 15). Group I served as normal control and group II (LD) and III (HD) were treated with sub-lethal concentrations of $1/15^{th}$ and $1/5^{th}$ of LC 50, respectively. After 96 h, fish from each group were weighed and sacrificed under anesthesia. Liver tissues were dissected out, weighed and processed for preparation of histological slides stained with H & E. Hepatic tissues were further studied for changes in hepatosomatic index and histopathological alterations. Data were statistically analyzed using Student's t- test. Acute exposure with hybrid pesticide significantly increased the HSI in high- dose group fish ($p<0.05$). Treatment with hybrid pesticide resulted in mild to moderate alterations in hepatic tissues in a dose dependent manner, such as, loosening of hepatic parenchyma, cytoplasmic and nuclear degeneration, hypertrophy of hepatocytes, fatty accumulation, sinusoidal alterations, activation of Kupffer cells, infiltration with leucocytes, congestion and disruption in central vein, hemorrhage etc. On account of hepatic toxicity induced by hybrid pesticide, effective control measures for its use and disposal should be adopted.

Key words : Chlorpyrifos, Cypermethrin, Hybrid, *Clarias batrachus,* Hepatocytes, Acute.

Use of pesticides in agricultural practices is a necessity for increasing the productivity of land and quality of crop products. However, their persistence in soil not only damages the composition of biotic and abiotic factors of soil, they can also reach nearby water bodies via surface runoffs, rain and drainage[10,12]. It increases the risk of exposure and accumulation of these toxicants in the aquatic organisms, including fish and thereby being a potential threat to human being also[27]. Among different types of pesticides, chlorinated organophosphates and synthetic pyrethroids are being commonly and extensively used in agricultural and aquaculture practices, industries and households[6]. Scientific study of effects of these pesticides on edible fish varieties have been an active area of research, like, exposure of Envoy 50 SC on *Heteropneustes fossilis*[2], Malathion on *Clarias batrachus*[4], Diazinon on fingerlings of Grass carp[24], 2,4-D on *Clarias batrachus*[19], Chlorpyrifos on common carp[15] and Cypermethrin on *Clarias batrachus*[18]. However, due to emergence of resistant varieties of pests and to increase the spectrum of action of pesticides, use of hybrid pesticides are in trend. One of the commonly used hybrid pesticides is combination of organophosphate chloryrifos and pyrethroid cypermethrin[10,12] and is available in market with different commercial names, viz, Anth-505, Turbo-505, Rips- 505, Bilbo B-505, *etc.* These two pesticides differ in their toxic potential and mode of action and they have been reported to have deleterious effects on the aquatic organisms as well as on human, when used singly[16,21,23,26]. However, reports say that the exposure with chlorpyrifos-cypermethrin combination pesticide can be more toxic than chlorpyrifos or cypermethrin alone for human as well as fish[5,7,9,22], as the organophosphate inhibits the detoxification process of pyrethroids[9]. But, not much literature is available in support of that. Falfushynska *et al*.,[5] reported that the health hazards of pesticide mixtures can not be predicted from the effects of single pesticide.

Fish have been used as an ideal animal model to study toxic effects of pollutants and toxicants on aquatic life[20]. These toxicants are metabolized and transformed into active metabolites in the liver. The involvement of liver in biotransfor-mation, metabolism and elimination of toxicants makes it susceptible for direct and immediate toxicity caused by them[8,10]. So, in this study, an effort has been made to investigate the effects of commercial hybrid pesticide formulation, Anth-505, a mixture of chlorpyrifos (50 %) and cypermethrin (5 % EC) on hepatic tissues of walking catfish, *Clarias batrachus*.

Test chemical :

Anth 505, a commercial grade hybrid pesticide containing Chlorpyrifos (50%) Cypermethrin (5% EC) was purchased from local market of Ranchi.

Experimental animal :

Live and healthy specimen of catfish *Clarias batrachus* (Linneaus, 1758) with average length of 20-25 cm and weight of 75-100 g were purchased from the local fish market (Hygiene fish market) in Ranchi, Jharkhand, India. Fish were maintained at the University Department of Zoology, Ranchi University, Ranchi in glass aquarium with 20

Litre of water. Fish were treated with 0.1% $KMNO_4$ to free from any dermal infections. They were kept for acclimatization in laboratory conditions for a period of two weeks prior to onset of experiment. The fish were fed with commercial pelleted fish feed and water in the aquaria was changed once daily. The average physico-chemical conditions of the laboratory and tap water in the aquaria were maintained throughout the study (Temperature- 25-30 , pH- 6.5-7.5, Photoperiod- 12h/12h light/dark).

Determination of dose :

Sub- lethal concentration of the test chemical to be administered was determined on the basis of previous work done by Kumar *et al.*,[12]. They reported the 96 hrs LC_{50} value of hybrid pesticide containing chlorpyrifos 50 % and cypermethrin 5 % EC on air- breathing fish, *Clarias batrachus* with average body size of 15-25 cm and body weight of 150-220 g to be 6µL/L of water. In the present study, two sub- lethal concentrations, *i.e.*, $1/15^{th}$ and $1/5^{th}$ of LC_{50} value were used for experimentation.

Experimental design :

After acclimatization, fish were randomly divided into three groups with five individuals in each. Fish were weighed before start of the experiment. Group I (NC) fish served as control and were maintained in normal tap water throughout the experiment. In group II (LD), fish were treated with sub-lethal concentration of 0.4 µL/L of water ($1/15^{th}$ of LC_{50}), whereas group III (HD) fish were intoxicated with sub- lethal concentration of 1.2 µL/L of water ($1/5^{th}$ of LC_{50}) of test chemical for 96 hours. During experimentation, the fish were maintained in the laboratory following guidelines of institutional ethical committee. On termination of the experiment, fish from each group were weighed and sacrificed under anesthesia. Liver tissues were dissected out, blotted free of blood, weighed and fixed in Bouin's fixative for further analysis.

Hepatosomatic Index (HSI) :

Hepatosomatic index was calculated using following formula:

$$HSI(\%\ of\ b.wt) = \frac{Weight\ of\ liver\ (g)}{Body\ Weight\ (g)} \times 100$$

Histopathology of liver :

After 24 hours of fixation, liver tissues from each group were transferred to 70 % ethanol. They were further processed for dehydration, clearing and embedding into paraffin wax. 5 µm thin sections were cut using microtome and stained with H & E following routine laboratory protocol for double staining. Photomicrographs were taken using image analyzer (Olympus CH20i) for histopatho-logical analysis.

Statistical analysis :

Data obtained were expressed as Mean ± Standard Deviation (SD). Difference between value of HSI in control and experimental groups were statistically analyzed using Students' *t*- test and were considered significant at $p \leq 0.05$.

Anatomical localization of liver :

The liver can be localized ventrally in the cranial/anterior region. It appeared bilobed

and slightly pinkish in colour (Fig. 1).

Effects of hybrid pesticide on HSI :

Acute exposure with low and high dosage of hybrid pesticide increased the hepato-somatic index of fish in group II and III (Table-1 and Fig. 2). However, the increase in HSI was significant in high- dose treated group (III) only, when compared with normal control group I ($p<0.05$).

Effects of hybrid pesticide on histology of liver :

Fig. 3 to 10 showed the photomicrographs of T. S. of liver of control and experimental group fish. In the control group, normal histological architecture of hepatic tissue was observed. Hepatic parenchyma was observed with cords of hepatocytes separated by narrow sinusoids (Fig. 3). Hepatocytes were polygonal cells with central, round nucleus, dark-stained nucleoli and abundant homogenous cytoplasm. In some hepatocytes, binucleated condition can also be observed. Central vein appeared normal with thin, intact endothelial lining. Kupffer cells can be observed along the sinusoidal lining (Fig. 4). No pathological signs, such as, distortion, congestion, dilation, necrosis, nuclear and cytoplasmic degradation and infiltration with inflammatory cells can be observed in liver sections of group I fish (Fig. 3 & 4).

To investigate the acute effect of Anth-505, a hybrid pesticide containing chlorpyrifos and cypermethrin, two sub- lethal concentrations; $1/15^{th}$ of LC_{50} and $1/5^{th}$ of LC_{50} were taken into consideration in the present study.

Acute exposure with low dose of hybrid pesticide ($1/15^{th}$ of LC_{50}) in group II fish showed considerate degree of pathomorphological changes in liver sections (Fig. 5-7). Loosening of hepatic parenchyma with loss of normal architecture can be observed (Fig. 5). Hepatocytes showed disruption in normal radiating arrangement. Many hepatocytes can be marked with deformed outline. Signs of nuclear degeneration, such as, shrinkage, condensation, dissolution and eccentric position can be marked in many hepatocytes. There was loss of nucleolus in some of the hepatocytes. Few binucleated hepatocytes can also be seen. Some necrotic regions showed presence of anuclear hepatocytes. There was presence of cytoplasmic vacuolation in the hepatocytes. Some patches of fatty accumulation can also be noted in the hepatic parenchyma (Fig. 6). In some regions, cloudy swelling of hepatocytes were apparent. After exposure with low dose of hybrid pesticide for 96 h, pathological conditions can be observed in the central vein also, such as, dilation, distortion, thickening of endothelial lining, congestion, infiltration with blood cells (Fig. 6 & 7). Mild Infiltration with leucocytes can be observed in hepatic parenchyma also. There was dilation in sinusoids. Lesser number of activated Kupffer cells was observed in sinusoidal space, as compared with those in normal control group fish liver (Fig. 6). In some regions, derangement and swelling of hepatocytes resulted in narrowing of sinusoidal space, thereby inhibiting the adequate blood flow towards central vein (Fig. 7).

In group III, fish were exposed to high dose ($1/5^{th}$ of LC_{50}) of Anth- 505, a hybrid pesticide containing chlorpyrifos (50%) and cypermethrin (5%). After 96 h, the hepatic

tissues showed pathological alterations similar to those in group II fish, but with greater extent of severity. The cloudy degeneration of hepatic parenchyma can be marked even at lower magnification (Fig. 8). Multiple strands of hemorrhage were notably present in liver sections of group III fish (Fig. 8 & 9). Although there was no appearance of congestion in central vein, distortion, rupture and thickening of endothelial lining was evident. Dilation (Fig. 9) as well as shrinkage (Fig. 10) can be observed in central vein. A considerable degree of derangement in hepatocytes and sinusoidal space can be seen in hepatic parenchyma of group III fish. Hepatocytes showed severe cytoplasmic and nuclear degeneration. Vacuolar as well as fatty degeneration of cytoplasm can be marked. Nucleus of hepatocytes showed variable necrotic changes, like, shifting to eccentric position, shrinkage and condensation (pyknosis) and dissolution (Karyolysis). In some areas, anuclear hepatocytes can also be seen. Contrasting conditions of sinusoid can be observed depending upon the arrangement and size of hepatocytes. In some regions, narrow sinusoidal space with multiple activated Kupffer cells can be seen (Fig. 9). Whereas, widening of sinusoidal space can be observed at some places (Fig. 10). A mild to moderate infiltration with leucocytes can be noted inside the central vein as well as in hepatic parenchyma (Fig. 9 & 10).

In the present study, toxicological effects of acute exposure with Anth-505, a hybrid pesticide containing chlorinated organophosphate, chlorpyrifos (50%) and synthetic pyrethroid, cypermethrin (5% EC) on histology of liver of freshwater fish, *Clarias batrachus* was investigated. For acute study, two sub- lethal concentrations, *i.e.*, $1/15^{th}$ (Low dose) and $1/5^{th}$ (High dose) of LC_{50} 96 h, was taken into consideration. Liver, being a prime organ responsible for metabolism and biochemical transformation of pesticides, showed compensatory as well as pathological alterations in its histological sections in response to stress caused by pesticide exposure. In this study, the condition of central vein, hepatocytes and sinusoids was explored in hepatic tissues of control and treated group fish. Following exposure with both the low (Group II) and high (Group III) dose of hybrid pesticide, central vein showed distortion and dilation (Fig. 5 & 7). Endothelial lining of central vein was ruptured and mild thickening can also be observed. Although the infiltration with blood cells was there in both the cases, congestion was present in central vein of liver of low dose group fish only (Fig. 6) and shrinkage of central vein was visible in high dose group fish (Fig. 10). Following exposure with hybrid pesticide, hepatic parenchyma lost its compactness and radiating arrangement as compared to control group liver. The extent of loosening was dependent on the dose of the pesticide administered. The cloudy appearance of hepatic parenchyma and derangement of hepatocytes and sinusoids was more apparent in high dose group fish than that in low dose group (Fig. 5 & 8). The severity of nuclear (shrinkage, karyolysis, pyknosis, loss of nucleus, eccentric position) and cytoplasmic (vacuolization, fatty accumulation, loss of cytoplasm) degeneration of hepatocytes was also directly proportional to the dose of hybrid pesticide used for treatment (Fig. 5-10).

Similar sort of pathological changes have been reported in hepatic tissues of different experimental fish treated with chlorpyrifos and cypermethrin alone. Corroborating with the present findings, a dose- dependent effect of

fatty degeneration, sinusoidal distortion, cytoplasmic degeneration, necrosis, nuclear changes such as karyolysis, karyorrhexis, karyopyknosis and eccentric nuclei, hypertrophy of hepatocytes, mild infiltration with lymphocytes, areas of congestion and hemorrhage was reported in *Oreochromis niloticus*[8,27], *Channa punctatus*[20], *Heteropneustes fossilis*[25], Juveniles of *Heteroclarias*[1] following exposure with chloryrifos. On the other hand, such histopathological alterations in hepatic tissue were observed in liver of *Channa marulius*[3], *Labeo rohita*[11], *Pangasianodon hypophthalmus*[13], *Clarias batrachus*[19] and *Cyprinus carpio*[14] following acute exposure with cypermethrin as well as in *Clarias batrachus*[18] and *Catla catla*[17] following chronic exposure with cypermethrin. In agreement with the findings of current investigation, the extent of histological alterations in hepatic tissues was directly dependent on the dose applied and the duration of exposure with pesticides under study. A dose- dependent increase in hepatosomatic index of fish following exposure with hybrid pesticide reported in the present study (Table 1, Fig. 2) was supported by Hossain *et al.*,[8] who documented an increase in hepatosomatic index in Nile tilapia exposed with chlorpyrifos. They further explained that the increased rate of necrosis following exposure with pesticide might have resulted in accumulation of extra mass in the form of fluid congestion, thereby increasing the weight of liver.

While comparing the toxic effects of chlorpyrifos and cypermethrin on *Cyprinus carpio*, Georgieva *et al.*,[6] reported the chlorpyrifos to be more toxic than cypermethrin and quoted that in combination they may exhibit synergistic cumulative effect. However, the availability of scientific literature regarding the effects of hybrid pesticide is meager. In the present investigation, commercial formulation of combination pesticide containing chlorpyrifos and cypermethrin has been used for acute study on *Clarias batrachus*. Similar to findings of current investigation, Kaur and Mishra[10] reported severe degeneration in hepatocytes (Cytolpasmic vacuolization, necrosis, nuclear pyknosis, diffusion and hypertrophy), congestion in central vein, fatty infiltration and dilation of sinusoids in *Channa punctatus* treated with hybrid pesticide containing chlorpyrifos and cypermethrin at the dose of $1/10^{th}$ of LC_{50} (96 h) for 30 days. They further explained that toxicant induced stress resulted in sudden withdrawal and utilization of stored glycogen from hepatocytes to meet the required energy demand leading to severe destruction of

Table-1. Effects of acute exposure with sub- lethal concentration of hybrid pesticide on hepatosomatic index of *Clarias batrachus*.

S. No.	Parameters	Groups		
		I (NC)	II (LD)	III (HD)
1.	Body weight (g)	93.33 ± 11.54	60 ± 17.32	60.00 ± 17.00
2.	Liver weight (g)	0.36 ± 0.07	0.29 ± 0.005	0.37 ± 0.02
3.	HSI (% of b.wt)	0.38 ± 0.07	0.51 ± 0.12	0.64 ± 0.11*

Values were mean ± standard deviation. *$p<0.05$, when compared with group I.

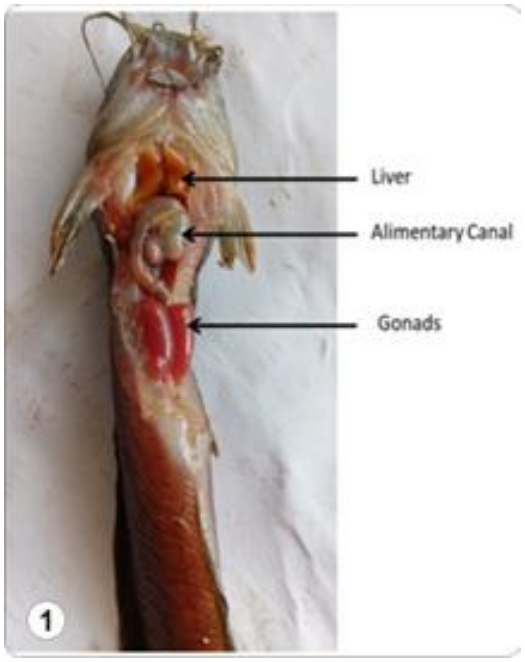

Fig. 1. Anatomical localization of liver in *Clarias batrachus*.

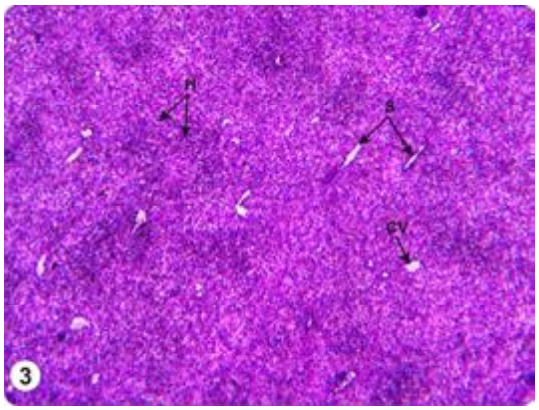

Fig. 3. Photomicrograph of T. S. of liver of normal control group (I) fish, *Clarias batrachus*, showing central vein (CV) and densely packed hepatic parenchyma with columns of hepatocytes (H) and sinusoids (S) (H&E; X100).

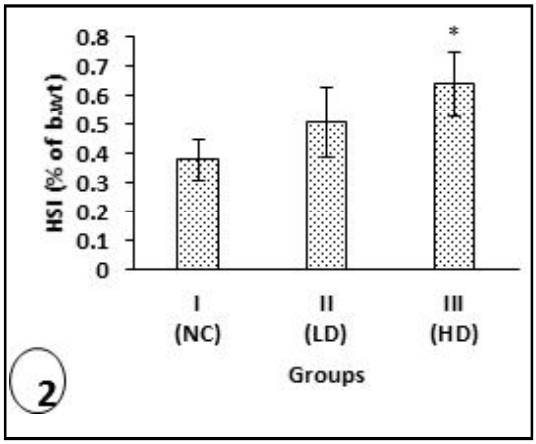

Fig. 2. Graph showing dose- dependent increase in hepatosomatic index of *Clarias batrachus* exposed to sub- lethal concentration of hybrid pesticide for 96 h.

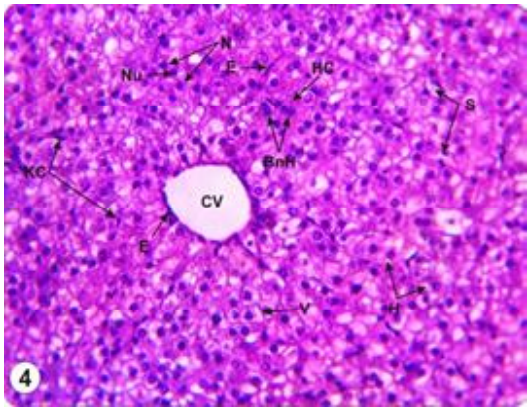

Fig 4: Photomicrograph of T. S. of liver of normal control group (I) fish, *Clarias batrachus*, at higher magnification showing normal central vein (CV) with intact, thin endothelial lining (E) and hepatic parenchyma with radiating cords of polygonal hepatocytes (H) with central, round nucleus (N), nucleolus (Nu) and homogenous cytoplasm (HC), binucleated hepatocytes (BnH), cytoplasmic vacuoles (V) and narrow sinusoids (S) separating the cords of hepatocytes, activated Kupffer cells (KC) in sinusoidal space (H&E; X400).

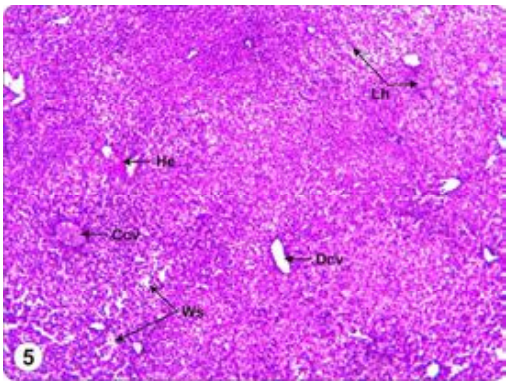

Fig. 5. Photomicrograph of T. S. of liver of group II fish, *Clarias batrachus*, treated with low dose (1/ 15th of LC50) of Anth- 505, a hybrid pesticide containing chlorpyrifos and cypermethrin for 96 h showing loosely arranged parenchyma of hepatic tissue (Lh) with derangement of hepatic cords and widening of sinusoidal space (Ws), dilation (Dcv) and congestion (Ccv) in central vein along with areas of hemorrhage (He) (H&E; X100).

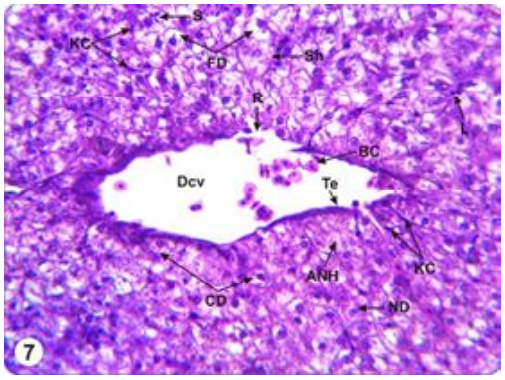

Fig. 7. Photomicrograph of T. S. of liver of group II fish, *Clarias batrachus*, following acute exposure with low dose of hybrid pesticide (Anth-505) showing dilation and distortion in central vein (Dcv), rupture (R) and thickening (Te) of endothelial lining, presence of blood cells (BC) in central vein, swollen hepatocytes (Sh) with cytoplasmic degeneration (CD), fatty degeneration (FD), signs of necrotic changes; anuclear hepatocytes (ANH), dissolution of nuclei (ND); narrowing of sinusoidal space (S), activated Kupffer cells (KC) and infiltration with leucocytes (L) (H&E; X400).

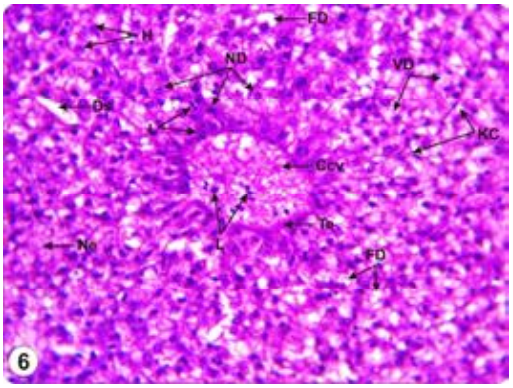

Fig. 6. Photomicrograph of T. S. of liver of fish, *Clarias batrachus*, showing marked histomorphological changes in hepatic tissue following acute exposure (96 h) with low dose of hybrid pesticide (1/15th of LC_{50}). The liver section of group II fish showed congestion in central vein (Ccv), thickening of endothelial lining (Te), hepatocytes (H) with nuclear (ND), vacuolar (VD) and fatty (FD) degeneration, areas of necrosis (Ne), dilation of sinusoidal space (Ds), activated Kupffer cells (KC) along with infiltration of leucocytes (L) inside lumen of central vein as well as in hepatic parenchyma (H&E; X400).

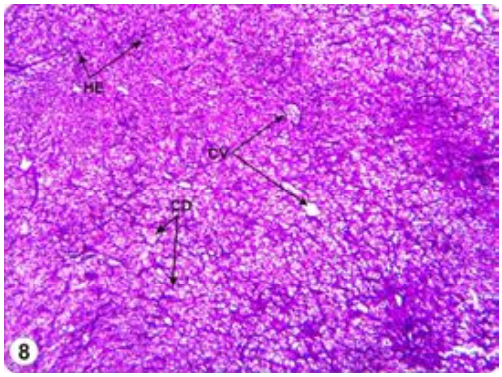

Fig. 8. Photomicrograph of T. S. of liver of group III fish, *Clarias batrachus*, exposed to high dose of hybrid pesticide; Anth- 505 (1/5th of LC50) for 96 h showing cloudy degeneration of hepatic parenchyma (CD), dilation and distortion in central vein (CV) and multiple strands of hemorrhage (He) (H&E; X100).

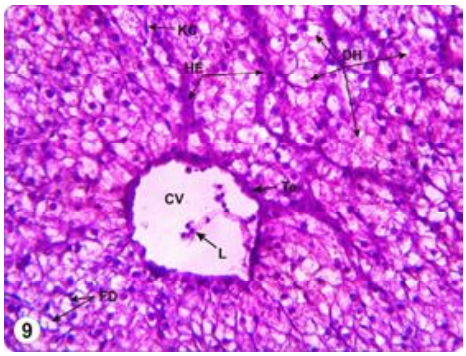

Fig. 9. Photomicrograph of T. S. of liver of group III fish, *Clarias batrachus* showing pathological alterations in hepatic tissue, such as, mild dilation in central vein (CV), distortion and thickening in endothelial lining (Te), swollen hepatocytes with severe cytoplasmic and nuclear degeneration (DH), fatty changes (FD), shrinkage of sinusoidal space, increased number of activated Kupffer cells (KC), hemorrhagic strands (He) and mild infiltration with leucocytes, following acute exposure with high dose ($1/5^{th}$ of LC_{50}) of Anth- 505, a hybrid pesticide containing chlorpyrifos and cypermethrin (H&E; X400).

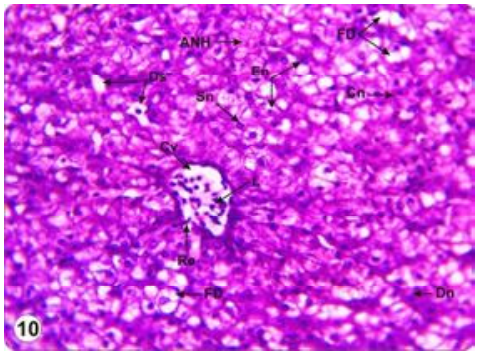

Fig. 10. Photomicrograph of T. S. of liver of group III fish, *Clarias batrachus*, showing severe pathological alterations in hepatic tissue following acute exposure with high dose ($1/5^{th}$ of LC50) of Anth- 505, a hybrid pesticide containing chlorpyrifos and cypermethrin. These changes can be marked as shrinkage and distortion of central vein (CV), rupture of endothelial lining (Re), loss of architecture of hepatic parenchyma, dilation of sinusoids (Ds), fatty degeneration of cytoplasm of hepatocytes (FD), necrotic changes in nucleus of hepatocytes (eccentric position (En), shrinkage (Sn), condensation (Cn) and dissolution (Dn)), anuclear hepatocytes (ANH) and infiltration with leucocytes (L) (H&E; X400).

hepatocytes and formation of intercellular spaces between them. Cytoplasmic vacuolization of hepatocytes reported in the present study might be a sign of metabolic damage due to degenerative processes and imbalance between rate of synthesis of molecules in the hepatocytes and their release into the circulation[10,14]. Neelima *et al.*,[14] also explained that focal necrosis of hepatic tissue might be due to elevated rate of detoxification to get rid of the toxic metabolites and inability of fish to make new hepatic cells. Furthermore, oxidative stress following exposure with pesticides can lead to cell death and necrosis[14,15].

While explaining the histological alterations in hepatic tissue of fish exposed to toxicants, Sharma and Jindal.,[17] suggested that the fish exhibited various compensatory responses such as dilation of sinusoids that can aid in facilitation of removal of toxicants from the hepatic parenchyma. In the present study, dilation as well as narrowing of sinusoidal space can be traced based on the condition of nearby column of hepatocytes. Swelling of hepatocytes caused narrowing of sinusoidal space (Fig. 7 & 9), whereas loosening and loss of hepatocytes resulted in widening of sinusoids (Fig. 6 & 10). Presence of lymphocytes and other blood cells in central vein as well as hepatic parenchyma and increase in frequency of activated Kupffer cells (Fig. 7 & 9) might be an indication of initiation of immunological

responses in terms of inflammation[17] and phagocytosis, respectively. Further, polymorphism in nuclear condition might be due to changes in nuclear lamina and alteration in expression of various nuclear transmembrane genes[17]. Higher concentration of toxicants can lead to nuclear hyperactivity[15], as evidenced by severity of nuclear damage in high dose group fish (Fig. 9 & 10). Hypertrophy of hepatocytes (Fig. 7 & 9) showed increased metabolic activity, whereas rupture and degeneration showed direct toxicity of the exposure with hybrid pesticide[15]. Fatty changes can be explained in terms of cellular response in the presence of lipophilic chemical agents and disturbances in metabolism of lipids[17]. Accumulation of lipids in vesicles restrict their interaction with other cellular components, thereby minimizing the damages and toxic effects[6]. Overall, a direct relation between the histopathological alterations in hepatic tissues and dose of the pesticide used can be established and subsequent metabolic dysfunctions can be predicted. However, the study further needs estimation of relevant biomarkers for metabolic status of liver to unveil the mechanism behind the toxicity caused by the combination pesticide.

Acute exposure with sub- lethal concentration of commercial grade hybrid pesticide, Anth- 505, containing chlorpyrifos and cypermethrin imposed mild to severe histological alterations in the hepatic tissue of *Clarias batrachus* as evidenced by loss of normal architecture of hepatic parenchyma, swollen hepatocytes, nuclear and cytoplasmic degeneration of hepatocytes, fatty accumulation, infiltration of leucocytes, widening or narrowing of sinusoidal space, damaged central vein, *etc*. As the hybrid pesticide is inducing deleterious effects on robust and hardy fish like *Clarias batrachus*, its noxious impact on other delicate aquatic organisms can be predicted. And being on top of the food chain, human becomes highly vulnerable for effects of biomagnification. So, effective measures should be taken regarding use as well as agricultural drainage of these pesticides to mitigate its exposure to aquatic life and life depending upon them.

Authors show sincere gratitude to Dr (Mrs) Suhasini Besra, Associate Professor, University Department of Zoology, Ranchi University, Ranchi for providing laboratory facilities and valuable guidance throughout the study.

Conflict of interest: Authors declare no conflict of interest.

Funding: None.

Contribution of authors

Aman kumar- Conception, design & analysis of data, drafting the article

Deepshikha samdershi- Analysis and interpretation of data, critical revision

Uzma Shamim- Maintenance of experimental set-up, Collection of data.

Priti Kumari- Maintenance of experimental set-up, Collection of data

All the authors approved the final version of the manuscript to be published.

References :

1. Abdulkareem S.I., M.O. Ajetunmobi and Y.O. Yekini (2020). *Tanzania Journal of Agricultural Sciences. 19*(2): 67-77.
2. Akter R., M.A. Pervin, and H. Jahan, *et al*. (2020). *JoBAZ. 81:* 47. https://doi.org/10.1186/s41936-020-00184-w.
3. Bhoi S., S. Patole and R.V. Karande

(2021). *Journal of Scientific Research of The Banaras Hindu University*. *65*(7): 90-93. https://doi.org/10.37398/JSR.2021.650719.

4. Diwakar A. and D. Pandey (2020). *International Journal of Basic Sciences and Applied Computing (IJBSAC)*. *2*(11): 1–3. https://doi.org/10.35940/ijbsac.K0162.0321120.
5. Falfushynska H., I. Khatib, and N. Kasianchuk, *et al*. (2022). *Sci Total Environ*. *833:* 155236. doi:10.1016/j.scitotenv. 155236.
6. Georgieva E., V. Yancheva, S. Stoyanova, *et al*. (2021). *Toxics*. *9*(6):125. doi:10.3390/toxics9060125.
7. Gupta B., S. Kerai and I. Khan (2018). *Indian J Anaesth*. *62*(11): 903-905. doi:10.4103/ija.IJA_338_18.
8. Hossain M.A., L. Sutradhar, T.R. Sarker, *et al*. (2022). *Saudi Journal of Biological Sciences*. *29*(7): 103316. https://doi.org/10.1016/j.sjbs.2022.103316.
9. Iyyadurai R., J.V. Peter, and S. Immanuel, *et al*. (2014). *Clin Toxicol (Phila)*. 52(5): 538-541. doi:10.3109/15563650.2014.909933.
10. Kaur G. and B. Mishra (2019). *International Journal of Biological Innovations (IJBI)*. *1*(2): 83-86. 10.46505/IJBI.2019.1209.
11. Khan N., M.S. Ahmad, S. Tabassam, *et al*. (2018). *Int. J. Agric. Biol. 20*(2): DOI: 10.17957/IJAB/15.0527.
12. Kumar R., R. Kumari and B.K.P. Mishra (2020). *J Entomol Zool Stud*. *8*(5): 1742-1745.
13. Monir Md S., M. Ashaf-Ud-Doulah, and K. Rahman Md, *et al*. (2016). *Asian J Med Biol Res*. *1*(3): 641-647. 10.3329/ajmbr.v1i3.26488.
14. Neelima P., C.L.A. Kumar, J.C.S. Rao and N.G. Rao (2015). *Int J Adv Res Biol Sci*. *2*(2): 34-40.
15. Pal S., E. Kokushi, J. Koyama, *et al*. (2012). *J Environ Sci Health B*. *47*(3): 180-195. doi:10.1080/03601234.2012.632285.
16. Sharma A., B. Yadav and S. Rohatgi, *et al*. (2018). *J Forensic Sci & Criminal Inves*. *9*(4): 555767. DOI:10.19080/JFSCI.2018.09.555767.
17. Sharma R. and R. Jindal (2020). *Environ Res*. *184:* 109359. doi:10.1016/j.envres.2020.109359.
18. Shrivastava R. and P. Thakur (2018). *HIGHBROW*. *5*(1): 69-76.
19. Soni R., S. Gaherwal and G. Shiv (2019). *Pramana Research Journal*. *9*(6): 1772-1781.
20. Stalin A., P. Suganthi, S. Mathivani, *et al*. (2019). *Environ Sci Pollut Res Int*. *26*(17): 17623-17631. doi:10.1007/s11356-019-05165-3.
21. Sunanda M., J.C.S. Rao, P. Neelima, K.G. Rao and G. Simhachalam (2016). *Int J Pharm Sci Rev Res*. *39*(1): 299-305.
22. Thanomsit C., C. Nualkaw, W. Prasatkaew, A. Wattanakornsiri, J. Nanuam, P. Nanthanawat and P. Kiatprasert (2020). *Thai J. Agric. Sci. 53*(3): 134-148.
23. Ullah S., A. Zuberi, M. Alagawany, *et al*. (2018). *J Environ Manage*. *206:* 863-871. doi:10.1016/j.jenvman.2017.11.076.
24. Vali S., N. Majidiyan, and D. Azadikhah, *et al*. (2022). *Water*. *14*(9): 1357. https://doi.org/10.3390/w14091357.
25. Verma D.K., R. Tripathi, V.K. Das and R.K. Pandey (2020). *The Scientific Temper*. *11*(1-2): 141-147.
26. Watts Meriel. (2012). Chlorpyrifos as a possible global POP. Pesticide Action Network. North America.
27. Zahran E., E. Risha, W. Awadin, *et al*. (2018). *Aquat Toxicol*. *197:* 47-59. doi:10.1016/j.aquatox.2018.02.001.