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Impact of sub-acute exposure to nuvan on serum lipoproteins of catfish, *Clarias batrachus* and their potential effects indirectly on human health

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Abstract

Pesticides are frequently applied in agricultural land, public health programs, and maintenance of trees or plants and also used as preservatives for different food materials for a long time. But these pesticides cause a lot of negative health and environmental impacts which affects the sustainability of an ecosystem. Among the different pesticides organophosphorus pesticide mostly used in agricultural purposes to prevent the disease causing agents like insects, pests, parasite due to its less persistence in our environment. Synthetic pesticide like Nuvan accumulations in the tissues of animals produce cyto-toxicity and have severed health issues due to its persistent nature. When these pesticides intake by aquatic animals specially fishes, they can interfere with the food chain. When such contaminated fishes eaten by someone they can destroy multiple systems in the body of human and other livings.

Changes in blood biochemical indices gives signs what is happening in body of animals after exposure of pesticides. Most edible and commercially valuable freshwater fish *Clarias batrachus* was used in this work for analyzed the hazardous nature of toxic pesticide nuvan on some biochemical parameters. When fish exposed to two sub-lethal levels of nuvan (0.0137 ml/L and 0.0274 ml/L) for 60 days showed that the pesticide induces changes in blood parameters. The results showed very highly significant ($p<0.001$) increase in Low- density lipoprotein-cholesterol (LDL) and highly significant ($p<0.01$) increase in Very low- density lipoprotein-cholesterol (VLDL) while very highly significant ($p<0.001$) reduction in high density lipoprotein- cholesterol (HDL) were noted in treated groups compared with the control. This study implicates that adverse effect on lipoproteins due to synthetic pesticides have a negative health effect as cardiovascular risk status. Research of environmental pollution and its adverse effects on human and other animals including fish is remarkable which can leads the sustainability of our environment.

Keywords: Synthetic pesticide; Nuvan; Catfish *Clarias batrachus*; Lipoproteins; Human health

1. Introduction

The edible things are only as safe and healthy as produce. There is pesticide residue in food and water. Nowadays, if we look at any edible thing available in market, is not without the use of any toxic chemical or pesticide. Pesticides and other toxic chemicals are also used in most of those substances through which the food can be preserved for a maximum time. Not only pesticides are hazardous to a person's health but also are dangerous to the environment. Pesticides are accumulates in our body, where they slowly but surely affect the body. When anyone eats a non-organic fruit, are also eats over 30 various pesticides that have been sprayed on it. Even if wash these fruits and vegetables, there are still more pesticides lingering on it and they could have seeped into the fruits or vegetables. Even packed drinking water, cold drinks and soft drinks are also combination of pesticides. Pesticides are chemicals that stop insects, weeds, and fungi from damaging crops. Agriculturists use them to increase the amount of crop productivity they are able to produce. Exposure to pesticides is also general in some workplaces and outdoors during crop spraying. Using pesticides in the

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soil or spraying crops with pesticides, can leave some residue on yield. Different pesticides can run off fields or soak through the ground to enter water resources.

Although all humans may consume pesticide residue, the Environmental Protection Agency (EPA) set a benchmark for harmless levels of pesticides in food. If a food product has an unsafe level of pesticides, the EPA can remove it from sale. When pesticides come in contacts with water bodies, they can interfere with the food chain and cause disease in hidden ways. Chemicals from pesticides get into water bodies, take them up by the aquatic animals. When humans eat such fishes (contaminated waters), they can damage multiple systems in the body of them. Biological cycling and Bioaccumulation of various pesticides in body of human and animals can cause numerous adverse health effects that have been associated with respiratory, carcinogenic, dermatological, neurological, gastrointestinal, endocrinal and reproductive effects [1, 2, 3]. However, WHO (World Health Organization) suggests that contact with large amounts of pesticides could affect reproduction and potentially be a cause of cancer [4]. Moreover 230 types of pesticides are used in India in which 24 types are used greatly and another 28 types of synthetic pesticides have been banned in India and many other countries due to their dense toxicity towards non target animals.

The environmental condition like temperature, pH and dissolved oxygen play significant role to increased pesticide toxicity in the presence of residual molecules. The organophosphorus pesticides are still highly toxic to the humans but their ability to decompose quickly in the environment decline their occurrence in freshwater and groundwater. Nuvan is a synthetic organophosphorus pesticide that has adverse effect on human health and aquatic environment. Nuvan or DDVP is used enormously in hospitals, transport and granaries and food processing elements and for disinfection during flight in aeroplane. It is not registered for direct application on any field-grown items [5]. For the action of nuvan the main mechanism is inhibition of acetylcholinesterase (AChE) by blocking synaptic transmission in cholinergic neurons with disruption of the nerve function in nervous system, resulting in impairment of metabolism of fats, carbohydrates and protein [6, 7]. Acetylcholinesterase is an enzyme that is significant for neurological function. It is also suggested that the random use of synthetic pesticides and fertilizers must be avoided for preserving our aquatic organisms. Nowadays findings related with nuvan pesticide also indicated acute aquatic toxicity in the water samples due to the higher concentrations of pesticide. Fish is consumed by mostly all parts of India and has proven to be a vector of hazardous elements as the literature suggests.

Keeping above facts in mind, the effect of widely used pesticide nuvan was taken up studying to find out acute and chronic effects in freshwater catfish *Clarias batrachus* as test animal. A voracious predator, *Clarias batratushas* has flat and broad head ability to walk across dry land with snake-like movements to use its pectoral fins, named the "Walking catfish". The biochemical content could be used as one of the non-specific bio indicator with regard to the effects of toxicants on living beings. Lipoproteins are round particles made of lipids (fat) and proteins (Figure- 1) that move in blood stream to body cells. Commonly known lipoproteins are high-density lipoproteins (HDL) and low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL). HDL or good cholesterol absorbs cholesterol from different body parts and delivers it back to the liver. The liver eliminates the cholesterol from our bodies. Both LDL and VLDL cholesterol contribute to plaque built-up in arteries and promote atherosclerosis [8]. However, having too any of them can put at more risk of medical situation like heart and liver diseases.

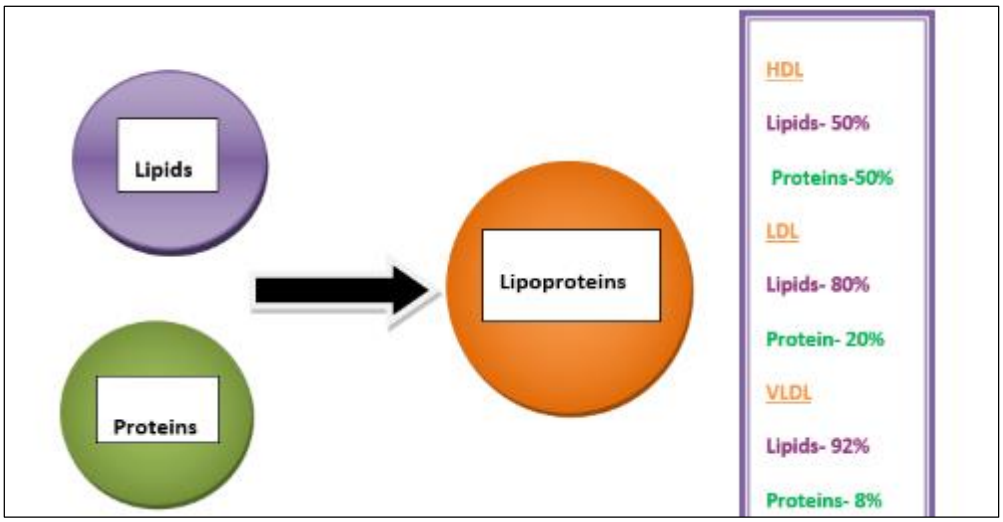


Figure 1 Lipoproteins

2. Materials and Methods

2.1. Animal maintenance

The freshwater catfish, *Clarias batrachus* (average weight 60 ± 10 g and 20 ± 5 cm length), were obtained from a local supplier from a local fish market, located in Agra (U.P.) and kept separately in glass aquariums that measured 75 cm by 37 cm by 37 cm. The fish were acclimatized for two weeks before experimentation in a laboratory environment when room temperature ranged between 18 ^{0}C-25 ^{0}C. Commercial fish food ingredients were feed to the fish daily at an average of 2.5% of their total body weight. For an ideal environment for the experimental fish, the water of the aquarium was changed every alternate day and draws off faecal matter and uneaten feed.

2.2. Toxicity Test

Commercial-grade formulation of synthetic organophosphorus pesticide (Nuvan- 2, 2- diclorovinyl diethyl phosphate) was used in this work because it is exclusively utilized in farming operations. Nuvan (Dichlorvos 76% EC) insecticide is manufactured by Insecticides (India) Limited purchased from the local market. The toxicity bioassay test of Nuvan was conducted for 96 hours to determine its median lethal concentration (LC_{50}).

The LC_{50} was calculated by probit analysis method [9]. The results of toxicity test in this study showed 50% mortality of test fish *Clarias batrachus* at 0.274 ml/L concentration during 96 hours exposure of nuvan. Mortality and behavioral alterations were also observed. For acute and chronic study test fish *Clarias batrachus* were exposed to different sub-lethal concentrations of nuvan- 0.0137 ml/L (1/20 of LC_{50}) for group (II) and 0.0274 ml/L (1/10 of LC_{50}) for group (III) over the period of 7, 15, 30, and 60 days respectively.

2.3. Blood Collection and Serum Separation

Blood samples for biochemical analyses were taken from cut caudal vein and collected immediately into serum separator tubes and then unattended for 20 to 40 minutes, in order to separate the serum. Then centrifuged the serum at 2500 rpm for 20 minutes after coagulation and kept at 20°C for future analysis of its biochemical characteristics. The serum or supernatant of test fish blood was used to assess various biochemical parameters; including HDL, LDL and VLDL. High Density Lipoprotein (HDL) activities in serum were determined according to Wybenga and Pilleggi [10] method. Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) were measured based on method suggested by Friedwald *et al*. [11]. All these biochemical analyses were conducted calorimetrically using spectrophotometer and purchased kits.

2.4. Statistical Analysis

Results were expressed as mean ±S Em. The intergroup variation data were analyzed statistically by employing R. A. Fisher's Test or F-Test or one-way analysis of variance (ANOVA). The level of significance was kept at 0.05 against the control (non-exposed) group.

3. Results

Alterations in lipoproteins showed a marked response to organophosphate insecticide activity showed in tables (1, 2, 3). The decrease of serum HDL and increase of serum LDL and VLDL in blood of the test fish exposed to Nuvan is clearly evident as per graphical presentation as in Figure (2, 3, 4).

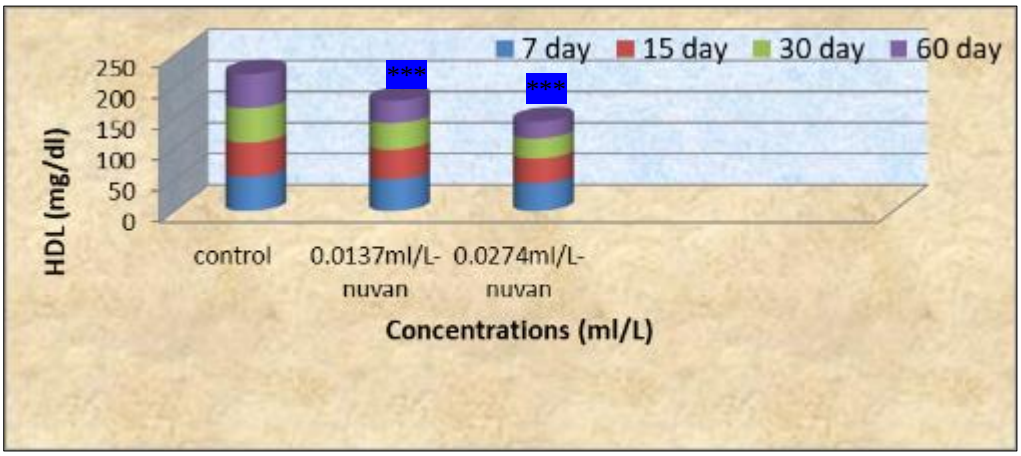
3.1. HDL (gm/dl) levels in blood serum of *Clarias batrachus* after exposure to Nuvan

When the test group (II) treated with 0.0137 ml/L (1/20 of LC_{50}) and group (III) with 0.0274 ml/L (1/10 of LC_{50}) of nuvan, then very highly significant ($P<0.001$) decrease were noted due to nuvan intoxication in serum high density lipoprotein (HDL) of test fish with increase in time of exposure (7, 15, 30, and 60 days). In control group (I) these values were not changed after whole period (Table- 1, Figure- 2).

Table 1 Alterations in blood serum HDL in fish *Clarias batrachus* exposed to nuvan pesticide

Concentration (ml/L)	HDL (mg/dl)	Exposure time				Significance level (ANOVA)
		7 days	15 days	30 days	60 days	
Control (Group– I)	Mean	55	55	55	55	Normal
	±S. Em	1.73	1.73	1.73	1.73	
Nuvan-0.0137 ml/L (Group– II)	Mean	51	47.5	42.5	36	Decrease (P<0.001)
	±S. Em	1.54	1.44	1.44	1.73	
Nuvan-0.0274 ml/L (Group– III)	Mean	44	40	32.5	27	Decrease (P<0.001)
	±S. Em	1.15	1.15	1.44	1.15	

(P<0.001)- Very highly significant



*** - Very Highly Significant

Figure 2 HDL levels in blood serum of the catfish *Clarias batrachus* exposed to nuvan pesticide

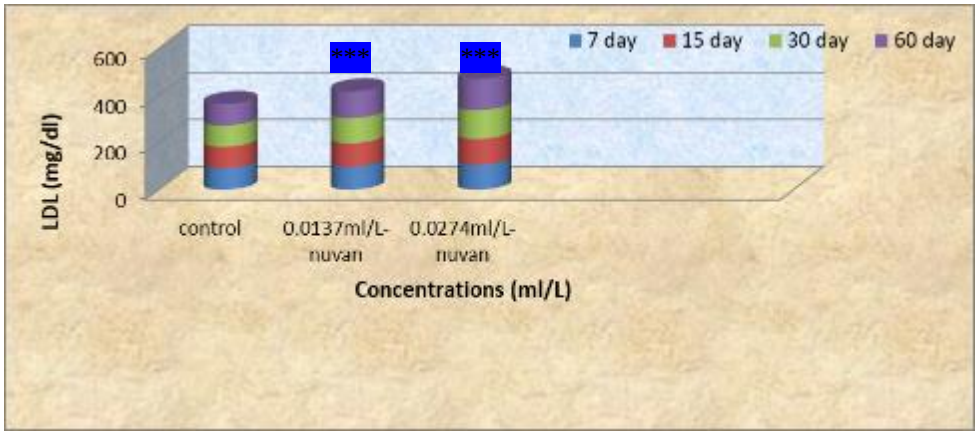
3.2. LDL (mg/dl) levels in blood serum of *Clarias batrachus* after exposure to Nuvan–

Statistically very highly significant (P<0.001) elevation were noted in the level of low-density lipoprotein (LDL) of test fish *Clarias batrachus* in group (II) after 0.0137 ml/L (1/20 LC_{50}) exposure and group (III) with 0.0274 ml/L (1/10 LC_{50}) of nuvan, with the increase in time of exposure (7, 15, 30, 60 days). But control group (I) not altered after whole experimental period (Table- 2, Figure- 3).

Table 2 Alterations in blood serum LDL in fish *Clarias batrachus* exposed to nuvan pesticide

Concentration (ml/L)	LDL (mg/dl)	Exposure time				Significance level (ANOVA)
		7 days	15 days	30 days	60 days	
Control (Group– I)	Mean	91	91	91	91	Normal
	± S.Em	1.73	1.73	1.73	1.73	
Nuvan–0.0137 ml/L (Group– II)	Mean	97	100.5	106.5	114	Increase (P<0.001)
	± S.Em	1.15	1.44	0.866	1.73	
Nuvan–0.0274 ml/L (Group– III)	Mean	105.5	110	122.5	133	Increase (P<0.001)
	± S.Em	1.44	1.73	2.02	1.15	

(P<0.001) – Very highly significant



*** - Very Highly Significant

Figure 3 LDL levels in blood serum of the catfish *Clarias batrachus* exposed to nuvan pesticide

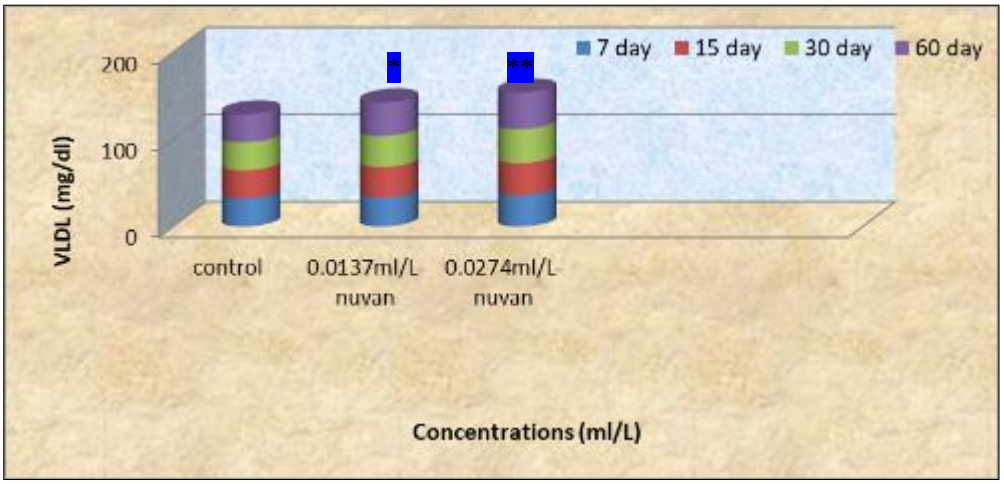
3.3. VLDL (mg/dl) levels in blood serum of *Clarias batrachus* after exposure to Nuvan–

Statistically significant (P<0.05) increase was noted in blood serum very low density lipoprotein (VLDL) of group (II) after 0.0137 ml/L (1/20 LC_{50}) of nuvan and highly significant (P< 0.01) increase at 0.0274 ml/L (1/10 LC_{50}) nuvan exposure were noted in group (III) with increase in exposure of time (7, 15, 30, and 60 days) while group (I) was not affected (Table- 3, Figure- 4).

Table 3 Alterations in VLDL in blood serum of *Clarias batrachus* exposed to nuvan pesticide

Concentration (ml/L)	VLDL (mg/dl)	Exposure time				Significance level (ANOVA)
		7 days	15 days	30 days	60 days	
Control (Group– I)	Mean	32.2	32.2	32.2	32.2	Normal
	±S.Em	1.15	1.15	1.15	1.15	
Nuvan-0.0137ml/L (Group– II)	Mean	33.2	35	35.8	38.4	Increase (P<0.05)
	±S.Em	1.15	1.15	2.02	1.73	
Nuvan–0.0274ml/L (Group– III)	Mean	35.8	37	39	42	Increase (P<0.01)
	±S.Em	1.15	2.59	1.15	1.73	

(P<0.05)- Significant; (P<0.01)- Highly significant



*- Significant, ** - Highly Significant

Figure 4 VLDL levels in blood serum of the catfish *Clarias batrachus* exposed to nuvan pesticide

4. Discussion

Organophosphates were found to be poisonous for freshwater fishes and other livings as evident in early observations [12, 13]. Biochemical parameters in various pathological and ecotoxicological analyses have been introduced as biomarkers of health status of animal. This analysis demonstrates that the sub-lethal concentrations of pesticides negatively affected the survival performance and the growth of test fish *Clarias batrachus*. Median lethal concentrations (LC_{50}) of nuvan in this work noted as 0.274 ml/L for freshwater catfish *Clarias batrachus*. Several other workers have analyzed median lethal concentrations (LC_{50}) of nuvan for different fish and noted the LC_{50} value of nuvan for most of fish may differ [14, 15, 16] and suggested that minute concentrations of synthetic pesticides can cause serious health issues in various fish. Behavioral dysfunctions in relation to the toxicity of synthetic pesticide in fish *Clarias batrachus* recorded were similar to other researchers [17, 18]. More studies were conducted regarding biochemical impacts of pesticides on fish but, the examined biochemical parameters like lipoproteins (HDL, LDL and VLDL) in blood serum has high variations in a certain species.

Lipoproteins are complex molecule consist of cholesterol esters and triglycerides surrounded with a central core by free cholesterol, phospholipids, and apolipoproteins, which accelerate lipoprotein formation and function. Because lipids (cholesterol and triglycerides) are insoluble in water these lipids fascinated in circulation conjoin with lipoproteins [19]. These lipoproteins play a most significant role in absorption and transport of dietary lipids by the small intestine, in the transport of lipids from the liver to peripheral tissues, and the transport of lipids from peripheral tissues to the liver and intestine (reverse cholesterol transport). Thus any variation in lipid metabolism affects the functioning of liver and the ability of fish to maintain energy that obtains nutrients and their immobility to survive in the long term. LDL or bad cholesterol makes up most of the body's cholesterol. A built up of LDL cholesterol can conges the arteries (atherosclerosis). These congestions can narrow the opening of the artery and initiate to a heart attack or stroke [20]. On the other hand, HDL or good cholesterol plays an important role in preventing from the development of atherosclerosis and also has a safe role in Cardio Vascular Diseases partly by the reversed transportation of cholesterol in which HDL deliver excess cholesterol to the hepatocytes for further excretion. Besides, HDL normally has a significant anti-inflammatory action in circulation and the arterial wall by the anti-oxidant enzyme located on the HDL particle.

In the present study LDL increases ($P<0.001$) and HDL reduces ($P<0.001$) in experimental groups of catfish *Clarias batrachus* and indicates liver insufficiency, malnutrition and heart problems. Likely LDL, VLDL levels also increased ($P<0.01$) after nuvan intoxication which can also produce inside the walls of blood vessels, which is undesirable [21]. It is also a main cause of CVD. Cardiovascular diseases (CVD) are one of the major health concerns at present time and contribute to the maximum mortality all over the world. More specially, when looking at lipid profiles individually, an extend amount of HDL and low amount of LDL prevents extra formation of cholesterol in the blood arteries and avoid health risk. Investigations on cholesterol and its role in heart hazards have focused on the blood serum activities of LDLs and HDLs. So the serum LDL: HDL ratio is a forecaster of heart disease risk than the blood serum cholesterol activities [22].

An elevation in the blood serum LDL and VLDL of *Clarias batrachus* in all treated groups were noted in this study when compared with the control. It might be caused severe destruction in hepatic tissues, which is the active site of lipid synthesis and storage. While HDL activity decreased very highly significantly, may cause of higher risk for stroke and heart disease. This work has similarity with the work of Rani [23] who denoted increment in LDL and VLDL levels, and decrement in HDL level in blood serum of *Channa punctatus* due to exposure of nuvan. Our finding gain support by some other investigators who have observed disturbances in fish lipoprotein under intoxication of pesticides. Blood and hepatic HDL, VLDL, LDL and cholesterol inhibited in *Channa punctatus* under stress throughout the experimental period observed by Das and Bhattacharya [24]. Similarly Yousef *et al.* [25] also recorded inhibition in HDL and significant increment in LDL and VLDL level after cypermethrin exposure. In the analyses of Celik *et al.* [26] range of HDL, LDL and VLDL were reported in black scorpion fish and suggested lipoprotein synthesized in the liver appeared to be associated with the lipid storage sites and other hydrophobic compounds in fish. This indicates organophosphorus pesticides have adverse effects on lipoproteins.

Kalender *et al.* [27] and Pothu *et al.* [28] suggested that one of the causes of increased total lipid levels due to the disturbance of carbohydrate metabolism appears to be cytotoxic effect on the pancreatic cells leading to relative deficiency of insulin. In decreased insulin level, most of the energy is derived from fats not from carbohydrates used as energy source. For needs of energy demands lipolysis occurs and the level of free fatty acids in blood is increased, which have not estimated in this research, resulting in increased serum lipid level [29]. Overstimulation of the nervous system activates energy demands resulting in the disruption of energy homeostasis that can lead to changed lipid metabolisms [30]. The article of Ullah and Zorriehzahra [31] focused on aspect of ecotoxicology and reviewed some major induced toxicological concepts of pesticides in fish including biochemical changes, behavioral changes, alterations in feeding

biology, histopathological damages, haematological alterations, vicissitudes in protein contents, fluctuations in acetylcholinesterase activity, induced genotoxicity, oxygen consumption and oxygen stress all over the world. In the review of Lushchuk *et al.,* [32], classified pesticides based on their use, nature, sources, physical state and patho-physiological effects and discussed.

There are many studies which state that elevated levels of persistent organic pollutants such as pesticides are associated with increased levels of serum lipids which are a major risk factor for cardiovascular disease [33]. If this association appears to be causal, it may have significant effects on any animal including human health. Tanvir *et al.* [34] investigated marked increase in LDL and VLDL with a concomitant reduction in HDL was observed in Chlorpyrifos treated rats [35] and concluded that chlorpyrifos bioaccumulation over time and exerts toxic effects on animals. Additionally, this pattern of change is closely related with a high-risk for coronary artery disease [36] due to the suggestive and antecedent role of LDL oxidation in the pathogenesis of atherosclerosis [37]. Moreover, low HDL may be attributed to nuvan induced hepatic damage, consequently in the impairment of lipoprotein synthesis. These findings suggest that prolonged exposure to nuvan may be a major risk factor for the occurrence of degenerative heart diseases [36]. There are some related studies which show that chronic exposure of pesticide leads to metabolic syndrome as shown by the one done by Mustieles *et al.* [38]. In the study of Pothu *et al.* [28] used non-HDL cholesterol and HDL (Total cholesterol ratio) as the measurements tools for risk factor assessment for cardiovascular status. Lipids undergo fast breakdown, re-synthesis and inter conversion. Hence it is important to study various lipid fractions in various tissues to provide a clear picture of lipid metabolism in action to pesticides which have not done in this study.

Increment in total lipid content suggested the lipogenesis under heptachlor pesticide intoxication in *Tilapia mossambica* reported by Radhaiah *et al.* [39]. Lipid contents increment mostly associated with increased bio concentration of lipophilic toxicants, which is frequently correlated enhanced toxicity of the *T. zilli* and the *Cyprinus carpio* under water pollution suggested by Adbelmeguid *et al.* [40]. Significant enhancement in cholesterol indicated lipid profile in blood hyperlipidanemia may be due to unmannered lipid metabolism which is probably the consequences of hepatic dysfunction and chronic hypoxic condition investigated by Logaswamy *et al.* [41]. Kumar and Gautam [42] analyzed the negative impact of nuvan on biochemical parameters of fish *Channa punctatus* and found increased levels of cholesterol, VLDL, triglyceride while the reduction in levels of HDL and LDL in the fish *C. punctatus.* Rao *et al.,* [43] reviewed toxicity and other toxic effects of nuvan on freshwater fishes and found adverse impacts on respiratory, behavioral and morphological activities. Acetylcholinesterase inhibition, biochemical alterations, chromosomal aberrations and carcinogenic effects, histopathological changes, haematological alterations were also investigated by them in freshwater fish. Significant reduction in high density lipoprotein- cholesterol (HDL cholesterol) and triacylglycerides in all treated groups compared to the control was also recorded by Esenowo *et al.,* [44]. These findings are opposed with those noted in previous investigation of Mahmood *et al.,* [45] in which they noted increase in HDL and decrease in LDL and VLDL levels in blood serum of *Labeo rohita* after exposed to Profenofos.

There are certain clinical trials on humans and other animals in which they exposed the subjects to various pesticides. Many more population-based observations have revealed possible relations between the organophosphates exposure and serious health effects including cardiovascular diseases [46], negative effects on the nervous system [47, 48] and on the male reproductive system [49], dementia [50], and also a possible high risk for non-Hodgkin's lymphoma [51]. Additionally, prenatal exposure to organophosphorus pesticides has been correlated with reduced gestational duration [52] and neurological problems mostly occurring in children [53]. The aim of Stefan *et al.* [54] study was to investigate the concentrations of POPs and other pesticides on high, low and very low-density lipoproteins (HDL, LDL and VLDL) in humans and the possible association with cardio vascular disease and cancer also occurrence in individuals living in a polluted area. Their analyses showed that pollutants concentrations in HDL were more connected with cardio vascular disease, while concentrations in LDL/VLDL were more connected with cancer. Due to pesticide exposure the population at high-risk of developing health issues includes normally pest control workers, farm workers or workers from agricultural industry, and the environmentally exposed individuals additionally residing near agriculture areas or the individuals exposed to household pesticides [55, 56]. Many other studies have also found that acute or chronic exposure to synthetic pesticides leads to airway diseases (asthma or allergic rhinitis). Pathak *et al.* [57] reviewed current status of pesticide impacts on human health environment, and its eco-friendly treatment as bioremediation and significance of finding potent microbes, novel genes, and biotechnological implementations for pesticide waste management to generate a sustainable environment. Ray and Shaju [58] collected data from the largest bodies of water, such as rivers and lakes, which contaminated by pesticide drift and discussed how hazardous pesticides are absorbed into body of fish and then how enter the food chain inducing dangerous impacts on human health when consumed by them. They were also noted that improper use of pesticides can negatively affect different levels of biological organization and all the component of environment.

5. Conclusion

Because of enormous use of pesticides in our foods, it is hard to safe any living being from the poisonous synthetic pesticides. The present work highlights that the observations regarding the use of lipoprotein profiles as biomarkers to assess synthetic pesticides exposure of freshwater species is really scare. The impact on fishes may be passed on to the other trophic levels impose a wider range of damages in an ecosystem. The results of present study revealed that a continuous application of pesticides and their combinations even at low concentration can have dramatic effects on fish health and indirectly on consumers. Since nuvan is more toxic it is suggestible not to apply pesticides on wet lands and keep pesticides out of water bodies and areas near waters. Adversely changes in biochemical contents will reduce the nutritional value of economically essential edible fish deteriorating their quality and simultaneously will become hazardous to the consumers caused by its bioaccumulation of the pesticide incorporated in it. Although most produce contains some level of pesticide residue, food testing ensures that the levels of pesticides low enough to not pose a risk to human health. Hence, synthetic pesticides must choose prudently and used in amalgamation with many other pest management tools, and safely appliances for targeting sustainable development and health protection of the consumers. Strong laws of the government also regulate the sale and use of pesticides for conserving our ecosystem.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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