

Research Article

Restorative effect of *Azadirachta indica* against fenvalerate induced haematological and biochemical toxicity in a freshwater fish *Clarias batrachus*

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Abstract

Pesticide use in recent times has increased many folds to enhance the yield of crops. The water bodies like ponds and others are extensively used for the cultivation of fishes. Since these fishes are a good source of protein and nutritious value, hence are consumed by humans. These pesticide-contaminated fishes are consumed by the human, causing health hazards. The objective of the present research work was to know the bio remedial effect of *Azadirachta indica* on fenvalerate induced toxicity in a freshwater air-breathing catfish "Mangur" known as *Clarias batrachus* (Linnaeus). The fishes were treated with 1/3rd of LC₅₀, i.e. 0.92 ppm of a pyrethroid pesticide fenvalerate and observed the fishes for 96 hours. Thereafter, the aqueous leaf extract of *Azadirachta indica* was administered orally, daily by gastric intubation method at the dose of 100 mg/Kg body weight per day for 15 days. The study revealed that, after the exposure of 0.92 ppm of fenvalerate, there was a significant decrease in RBC count from 2.76 to 2.14 X (10⁶) (mm⁻³), Total leukocyte count from 18.10 to 16.30 X (10³) (mm⁻³), packed cell volume from 24 % to 16% , haemoglobin from 6.89 g/100 ml to 4.23 g/100ml of blood whereas increase in the level of serum glucose and cholesterol level from 50.33 mg/dl to 103.73 mg/dl and 168.52 g/dl to 248.99 g/dl respectively. At the same time, serum protein level decreased from 3.16g/dl to 2.52 g/dl due to pesticide in the experimental fishes. But, after the administration of aqueous leaf extract of *Azadirachta indica* there was significant normalisation in the test parameters of the fishes. The study indicated that neem leaves possessed restorative effect against fenvalerate induced toxicity in *Clarias batrachus*.

Keywords: Fenvalerate, *Azadirachta indica*, Haematology, Biochemical assay, *Clarias batrachus*

INTRODUCTION

Bihar is the agriculturally based state, which is typically based on the cultivation of various types of Kharif and Rabi crops. For a better yield of crops, pesticides are widely used by the farmers. In recent times, fish farming in the state has attracted a lot of farmers. These fishes are cultivated near the agricultural land in the aquatic bodies. The aquatic bodies such as rivers, ponds also receive lots of agricultural runoff water which contains pesticides. These pesticides in higher concentrations are causing toxicity in the water fauna of the ponds, especially the fishes. Humans have been consuming these fishes due to its high nutritious value. The fishes through biomagnification of the pesticides are causing health hazards to humans. The organochlorine pesticides are more toxic than organophos-

phates. Synthetic pyrethroids have been known as safe and environmentally friendly due to their mild toxicity to insects, mammals and birds, and they are reported highly toxic to a number of other non-target organisms especially aquatic organisms including fishes. Fenvalerate pesticide is capable of existing together in harmony with several other pesticide group and chemicals used to modify plant growth and plant micronutrients (Tomlin, 1995; Elbert *et al.*, 2005; Bretschneider *et al.*, 2007, Caglayan *et al.*, 2016). It causes very least impact on the mammalians but possesses very high insecticidal activity. Fenvalerate is a known neurotoxic pesticide which disrupts the sodium channels of neurons of insects followed by damaging the gamma-aminobutyric acid receptors and ATPase pathways (Narahasi, 1983; Cole *et al.*, 1984; Clark and Matsumura, 1982; Matsu-

mura, 1983; Eells *et al.*, 1993). Indiscriminate use of fenvalerate pesticides in agricultural practices has resulted in accumulation in vital tissues of fishes (Coats *et al.*, 1989; Mishra, 1996). The toxicological evaluation of fenvalerate has been carried out by various researchers (Lee *et al.*, 1985; Tripathi, 1992; Tripathi and Verma, 2004; Jigyasu and Paul, 2016).

A new approach for the treatment of toxicity through medicinal plants has been very much popular in the recent times as they have proven a promising role to control the problem (Dutta-Mitra and Ahmed, 2015; Jha and Paul, 2020; Kumari and Paul, 2020). To enhance the fish immunity and to control the toxic effects of pesticides various medicinal plants have been widely used by the fish farmers (Vallejos *et al.*, 2016; Awad and Awad, 2017; Nhu *et al.*, 2020). All the parts of neem (*Azadirachta indica*), a member of the Meliaceae family is known as an excellent medicinal plant showing important role as health -promising effect due to its antioxidant source. Leaves mainly contain quercetin (flavonoid) and nimbosterol (β - sitosterol) as well as a number of limonoids (nimbin and its derivatives). Quercetin (a polyphenolic flavonoid) is known to have antibacterial and antifungal properties. The principal constituents of neem leaves include protein (7.1%), carbohydrates (22.9%), minerals, calcium, phosphorus, vitamin C, carotene etc. But they also contain glutamic acid, tyrosine, aspartic acid, alanine, praline, glutamine and cystine like amino acids, and several saturated and unsaturated fatty acids) (Sujarwo *et al.*, 2016; Gupta *et al.*, 2017).

Neem is widely used for the treatment of skin manifestations, skin lesions, ulcers, urinary tract problems, gastrointestinal problems, diabetes etc. (Pandey *et al.*, 1994; Subapriya and Nagini, 2005; Manikandan *et al.*, 2008; Neem, 2012; Sharma *et al.*, 2014).

The objective of the present investigation was to evaluate the toxic impact of fenvalerate, a commonly used insecticide, on haematological and biochemical parameters of a freshwater air-breathing fish *Clarias batrachus* and to find the restorative effect of medicinal plant, *Azadirachta indica* L. (neem) against fenvalerate induced toxicity.

MATERIALS AND METHODS

Live specimens of *C. batrachus* were procured from the local market of Patna, Bihar (India) and were acclimatized in the laboratory before experimentation. The proper condition was maintained for 15 days to acclimatize the fishes in aquaria. The fishes were fed with a mixture of egg and chopped wheat (flour) /suji with a very little amount of starch. The fishes were also fed with sliced goat liver every alternate day during mid-day. Utmost care was taken to keep the animals healthy and disease-free animals for the experiment. The experiments were done in the Post Graduate Department of Zoology, Patna University, Patna, Bihar, India. To conduct experiments, the ethical approval

was given by Post Graduate Departmental Council of Zoology, Patna University, Patna, Bihar.

Fenvalerate 20% (Isagro-Asia, Gujarat, India) was procured from Patna market, and fishes were treated with it in the water contained in an aquarium at the dose of 1/3 of LC₅₀, i.e. 0.92 ppm and observed for 96 hrs.

To prepare an extract of leaves of neem were collected from the tree present in Patna Science College, Patna University and aqueous extract of neem leaves obtained using the method adopted by Neogi *et al.* (2007). LD₅₀ of leaf extract was estimated as 600 mg/Kg body weight. The aqueous extract of neem at the dose of 100 mg/Kg body weight of the fish per day was given orally to the fenvalerate treated fishes daily.

A set of 10 fishes were considered as control group. The control group of fishes received no treatment. The second group of 10 fishes were treated with fenvalerate(0.92 ppm) for 96 hours. The third aquarium fishes treated with fenvalerate insecticide were administered neem extract in the prerequisite dose, i.e. 100 mg/Kg body weight of fishes per day orally for 15 days. The haematological indices such as red blood cell count, total white blood cell count, packed cell volume, haemoglobin percentage level and differential counts viz. eosinophil, lymphocytes and monocytes were estimated by the methods suggested by Darmady and Davenport (1954).

For the study of biochemical parameters, serum from the fish blood was extracted, and tests were carried out for the analysis of the total glucose levels, total protein levels and total cholesterol levels as per the methods suggested by Godkar and Godkar (2006).

Statistical evaluation of the results was done by adopting one-way ANOVA and Dunnett's test. Results are shown as mean and standard deviation.

RESULTS AND DISCUSSION

In the fenvalerate treated fishes the decreased value of haematological indices such as RBC counts ($\times 10^6\text{mm}^{-3}$), TLC counts($\times 10^6\text{mm}^{-3}$), differential counts ($\times 10^6\text{mm}^{-3}$), packed cell volume (%), haemoglobin (g/100 ml), eosinophils (%) and monocyte levels(%) whereas increase level was observed in lymphocytes (%) after the fenvalerate exposure to the fishes. The restorative effect in haematological parameters was seen when the fishes were fed with the neem leaf extract for two weeks (Table 1).

During the biochemical study of fishes increased level of serum glucose and serum cholesterol of fishes was observed whereas serum protein level showed increased manner due to fenvalerate treatment in comparison to control fishes. Again, there was an ameliorating effect in the altered values of biochemical indices after the treatment of neem extract for 15 days (Table 2).

The pesticides have caused serious health hazards to aquatic life. The biomagnification of the pesticides

Table 1. Mean value of Hematological parameters of *C. batrachus* exposed to sublethal concentration of fenvalerate (0.92 ppm) for 96 hours and neem extract for 15 days.

Parameter	Control	Fenvalerate treated for 96 hours	Neem treated for 15 days
RBC ($\times 10^6 \text{mm}^{-3}$)	2.76 \pm 0.032	2.14 \pm 0.023	2.59 \pm 0.154
Total Leukocyte Count ($\times 10^3 \text{m}^{-3}$)	18.10 \pm 0.015	16.30 \pm 0.074	17.60 \pm 0.126
Differential Count ($\times 10^6 \text{mm}^{-3}$)	12 \pm 0.230	09 \pm 0.083	11 \pm 0.073
Packed Cell Volume (%)	24 \pm 0.189	16 \pm 0.041	22 \pm 0.290
Haemoglobin (g/100ml)	6.89 \pm 0.031	4.23 \pm 0.073	5.20 \pm 0.189
Eosinophils (%)	78 \pm 0.027	64 \pm 0.061	74 \pm 0.470
Lymphocytes (%)	10 \pm 0.152	16 \pm 0.091	12 \pm 0.241
Monocytes (%)	01 \pm 0.039	Zero	0.4 \pm 0.056

Table 2. Mean values of biochemical parameters of *C. batrachus* exposed to sublethal concentration of fenvalerate (0.92 ppm) for 96 hours and neem extract for 15 days.

Parameter	Control	Fenvalerate treated for 96 hours	Neem treatment for 15 days
Serum Glucose (mg/dl)	50.33 \pm 0.103	103.73 \pm 0.208	59.98 \pm 0.173
Serum Cholesterol (g/dl)	168.52 \pm 0.319	248.99 \pm 0.394	203.73 \pm 0.301
Serum Protein (g/dl)	3.16 \pm 0.009	2.52 \pm 0.006	2.89 \pm 0.007

through the food chain reaches humans. This leads to the entry of pesticidal toxicity to the vital organs of the human body leading to cause disease in them (Katagi, 2010; Clasen *et al.*, 2018; Yang *et al.*, 2020; Mojiri *et al.*, 2020). The pollution in the aquatic fauna can be observed in the fishes as they are the best indicators. Hence their haematological parameters can be used for the toxicity evaluation (Sharma and Singh, 2004 and 2006; Ramesh *et al.*, 2014; Woo *et al.*, 2018; Bojarski and Witeska, 2020).

In the present haematological study, there was a significant decrease in the RBC counts, TLC counts, Differential counts, Haemoglobin percentage, PCV percentage, Eosinophils and Monocytes percentage after fenvalerate exposure while after neem extract treatment there was a significant decrease in their levels. There was a significant increase in the Lymphocyte percentage after Fenvalerate exposure, but in neem leaf extract-treated group, there was a significant decrease in their levels.

Fenvalerate has caused significant damage on *C. batrachus* on the haematological parameters. The significant decrease (from $2.76 \pm 0.032 \times 10^6 \text{mm}^{-3}$ to $2.14 \pm 0.023 \times 10^6 \text{mm}^{-3}$) in RBC indicated that erythropoietic stem cell damage due to fenvalerate, while decrease in the total leukocyte counts (from $18.10 \pm 0.015 \times 10^6 \text{mm}^{-3}$ to $16.30 \pm 0.074 \times 10^6 \text{mm}^{-3}$) was due to myelosuppression. Ultimately it caused low immunity and anaemia in the pesticide-treated fish (Narra, 2016; Narra *et al.*, 2017; Abd El-Rahman *et al.*, 2019). Furthermore, the increased value of lymphocytes denoted the obvious defence breach in the fish body (Gul *et al.*, 2012).

During experimentation, there was increased value in serum glucose levels from 50.33 mg/dl to 103.73 mg/

dl and serum cholesterol value from 168.52 g/dl to 248.99 g/dl while decrease value in the serum protein from 3.16g/dl to 2.52 g/dl in biochemical parameters of fishes due to exposure with fenvalerate. The fats are stored in the form of cholesterol and glucose in the form of glycogen in the liver. The hepatotoxicity caused due to fenvalerate has led to the change in biochemical parameters. Hence, change in the levels denotes the impact of toxicity. (Binukumari *et al.*, 2016; Nejatkhah Manavi *et al.*, 2018; Vieira and Dos Reis Martinez, 2018; Pico *et al.*, 2019).

In the present study, the aqueous extract of neem leaves was used as an antidote against the fenvalerate induced toxicity in *Clarias batrachus*. There was a significant increase in the haematological parameters such as RBC counts, Total leucocyte counts, Differential counts, Packed cell volume percentage, haemoglobin percentage, Eosinophil percentage and Monocyte percentage while a decrease in the Lymphocyte percentage against fenvalerate induced toxicity. This denotes that the haematopoietic stem cells are significantly revived due to quercetin (flavonoid) and nimbosterol (β - sitosterol), limbin and its derivatives which are active constituents of neem. The active ingredients quercetin, a flavonoid and nimbosterol (β - sitosterol) play the vital role as antioxidant and immuno-booster. This enhances the defence mechanism of the fishes such as *Channa punctatus* Lates *calcarifer*, *Pangasianodon hypophthalmus*. Moreover, they are also helpful to eliminate the pesticide bioaccumulation from the fish body (Farah *et al.*, 2006; Talpur and Ikwannuddin, 2013; Nhu *et al.*, 2019^a).

In the present study, neem leaf extract played a vital role to control the fenvalerate induced toxicity in *C.*

batrachus. The normalisation of haematological and biochemical parameters of the fishes at much extent denotes the active function of the quercetin and nimboesterol particularly against pathogen *Aeromonas hydrophila* in goldfish, zebrafish (*Danio rerio*). (Harikrishnan and Balasundaram, 2008; Batista *et al.*, 2018; Kapinga *et al.*, 2018; Nhu *et al.*, 2019^b). Hence, Neem extracts can be used as an antidote against fenvalerate induced toxicity in *C. batrachus*.

Conclusion

Fenvalerate caused a deleterious effect on the fishes at the haematological and biochemical level such as RBCs, total leucocytes, haemoglobin, serum glucose, serum cholesterol, serum protein in the indigenous fish *C. batrachus*, common food item of the people. *A. indica* (neem) played a vital role to control the fenvalerate induced toxicity. Hence, it possessed the restorative activity in fenvalerate induced toxicity. The aquatic extract of the different parts of neem trees, easily available in India, may be used for therapeutic uses of several diseases of the fishes caused due to common pollutant pesticides in the aquatic bodies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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