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Research Article

Protective effect of ascorbic acid against fenvalerate induced toxicity in air-breathing fish *Clarias batrachus*

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Abstract
The food demand in recent times has increased many folds. In order to augment the need for food, the agriculture practice is extensively carried out by the farmers and pesticides are widely used by them for the better yield of crops. Fishes are also cultivated by these farmers, and humans are consuming the fishes as they are very high nutritious food product. But, these pesticides through agricultural runoffs are contaminating the ponds as well the aquatic fauna like fish. The present research work deals with the evaluation of the protective effect of ascorbic acid on fenvalerate induced nephrotoxicity in *Clarias batrachus.* The pyrethroid pesticide- Fenvalerate EC 20% was administered directly in the water contained in the aquarium at the dose of 0.027 ppm, 0.042 ppm and 0.083 ppm respectively for 96 hrs hours after the dose calculation through LC_{50}. Thereafter, ascorbic acid was administered orally by gastric intubation method at the dose of 200 mg/Kg body weight per day for 04 days to each pesticide treated group. The study revealed that, after the exposure of fenvalerate, there was significant damage at the biochemical levels like urea, creatinine, protein and albumin and histopathological study of kidney tissue in fish *C. batrachus*. But, after the administration of ascorbic acid, there was a significant restoration in the biochemical levels and in histopathology of the kidney of fish. The study concluded that Ascorbic acid possessed protective effect against fenvalerate induced toxicity in *C. batrachus*.

Keywords: Ascorbic acid, Biochemical assay, Fenvalerate, Histopathological study

INTRODUCTION

India is an agriculture-based country with the cultivation of all types of crops. For the better yield of crops, farmers are extensively using the pesticides. This has led to the entry of these agrochemicals in the human food chain. Apart from this, the humans are also consuming fish products because of its high nutritious value. These fishes are cultivated in the ponds which are near the agricultural fields. This has caused the migration of agrochemicals into the ponds by agricultural runoffs contaminating the aquatic fauna of the ponds. These fishes if consumed by humans, can cause serious health hazards. Organochlorine pesticides are known to cause more damage than organophosphates. But, in recent times the synthetic pyrethroids are known to be much safer and eco-friendly as they have the least toxicity to other animals but selective toxicity to insects. Fenvalerate is compatible with many other pesticides, plant growth regulators and micronutrients. But, causes the least toxicity to humans but possesses very high insecticidal activity (Coats *et al*., 1989; Tripathi, 1992; Elbert *et al*., 2005; Bretschneider *et al*., 2007).

Fenvalerate is a pyrethroid group of pesticide and is highly neurotoxic to the insects which damage the sodium channels. Moreover, the neurotransmission is severely disrupted especially the gamma-aminobutyric acid receptors and ATPase pathways (Clark and Matsumura 1982; Matsumura 1983; Narahashi, 1983; Cole *et al*., 1984; Eells *et al*., 1993; Zhang *et al*., 2017; Awoyemi *et al*., 2019). The extensive use of pesticides has led to the accumulation of these in the fish muscles as well as its vital tissues. The impact of fenvalerate on fish metabolism has been carried out by a plethora of researchers (Lee *et al*., 1985, Tripathi, 1992; Tripathi and Verma, 2004; Velmurugan *et al*., 2007; Ma *et al*., 2009, Jigyasu and Paul 2016).

In recent times, researchers have focussed their work on the development of antidotes against the pesticide-induced toxicity in fish. This not only controls the prob-

lem but also enhances the protein quality and immunity of the fish (Datta-Mitra and Ahmed 2014; Awad and Awad 2017; Vallejos *et al*., 2016; Nhu *et al*., 2019). Ascorbic acid, also known as Vitamin C is a natural water-soluble vitamin with potent antioxidant, antibacterial, antitoxic, immune-booster and many other activities which are helpful in the metabolic functions of the body. They are exclusively found in the citrus fruits, vegetables etc. (Merlevede 1950; Fujimoto *et al*., 2013). Hence, ascorbic acid has proven very promising role to control the disease in pesticide-induced toxicity (Bhattacharya and Kaviraj 2009). The objective of the present study was to evaluate the toxic impact of fenvalerate, a commonly used insecticide on biochemical (Kidney Function Tests) parameters and histopathological study of an air-breathing fish *Clarias batrachus* and to find the ameliorating effect of ascorbic acid against fenvalerate induced toxicity.

MATERIALS AND METHODS

Animals: The experiment was carried out in the Department of Zoology, Patna University, Patna, Bihar, India. The ethical approval was obtained from the Post Graduate Research Council of Patna University, Patna, Bihar. Live specimens of *C. batrachus* were procured from the local market of Patna, Bihar (India) and were acclimatized in the laboratory before experimentation. The fishes were captivated in large aquaria with 50gallon water capacity. The fishes were fed with traditional lab food as chopped goat liver and earthworms. The experimental animals were taken care throughout the experimental period so as to keep them healthy and free from parasites.

Test chemical: In the present study, Fenvalerate 20% (Isagro-Asia, Gujarat, India) was procured from the local market of Patna, Bihar, India. Fenvalerate was administered directly in water contained in three aquariums with the different doses of Fenvalerate 0.027 ppm, 0.042 ppm and 0.083 ppm respectively after the dose calculation through LC_{50} (2.75ppm).

Preparation of dose of ascorbic acid: Ascorbic acid (Celin-500 mg) from Galaxo Company was used and 200 mg/kg b.w. was calculated as maximum permissible dose after LC_{50} calculation. The dose was administered by gastric intubation method to the fishes (Vale and Kulig 2004).

Study groups and sampling: The control group of fish received no treatment. The 'treatment' groups received fenvalerate at the dose of 0.027 ppm, 0.042 ppm and 0.083 ppm for 96 hrs in the respective aquariums. Upon all the three fenvalerate treated groups, Ascorbic at the dose of 200 mg/Kg body weight was administered to the respective groups of aquariums for 04 days by gastric intubation method to the fishes.

Biochemical evaluation: For the biochemical study, serum from the fish blood was extracted and tests were carried out. In this study, the serum urea levels (Berthelot, 1859), serum creatinine levels (Bonsnes 1945), serum protein levels (Gornall *et al*., 1949) and albumin levels (Doumas *et al*., 1971) were evaluated.

Histopathological evaluation*:* For the histopathological study, kidney tissues were dissected and collected from all the group of sacrificed fishes. The tissues were washed in 0.65% normal saline, grossed into small pieces and finally fixed in 10% neutral formalin for 24 hours. Thereafter, tissues were dehydrated through the process of graded series of ethanol and finally embedded into paraffin. Thin sections of 5µm thickness were sliced through rotary microtome and stained with haematoxylin and eosin (H&E) for the histopathological study under light microscope (Gamble 2008).

Statistical analysis: The results are presented as mean ± SD, and the total variation present in a set of data was analysed through one-way analysis of variance (ANOVA). The difference among mean values has been analysed by applying Dunnett's test. Calculations were performed with the Graph Pad Prism Program (Graph Pad Software, Inc., San Diego, U.S.A.). The criterion for statistical significance was set at $P<0.05$.

RESULTS

Biochemical evaluation: In the present study, there was significant ($p<0.0001$) increase in the levels of serum urea in control as 16.67±0.9545, fenvalerate 0.027ppm treated as 59.93±2.442 with significant ($p<0.0001$) normalisation in ascorbic acid 200mg/kg b.w treated group as 29.83±2.469, fenvalerate 0.042ppm treated as 48.0±1.844 with significant ($p<0.0001$) normalisation in ascorbic acid 200 mg/kg b.w. treated group as 40.17±2.301, fenvalerate 0.083ppm treated as 43.67±1.542 with significant normalisation in ascorbic acid 200 mg/kg b.w. treated as 29.83±2.469 (Fig. 1).

In serum creatinine analysis the level in control was 0.766±0.032 fenvalerate 0.027ppm treated was 1.992±0.075 with significant ($p<0.0001$) normalisation in ascorbic acid 200mg/kg b.w treated group was 0.916±0.035, fenvalerate 0.042ppm treated was 1.475±0.038 with significant normalisation ($p<0.05$) in ascorbic acid 200mg/kg b.w. treated group was 1.033±0.057, fenvalerate 0.083ppm treated was 1.275±0.038 with significant ($p<0.0001$) normalisation in ascorbic acid 200mg/kg b.w. treated was 1.133±0.062 (Fig. 2).

In serum protein analysis, the level in control was 4.383±0.185 fenvalerate 0.027ppm treated was 13.43±0.639 with significant ($p<0.05$) normalisation in ascorbic acid 200mg/kg b.w. treated group was 6.317±0.235, fenvalerate 0.042ppm treated was

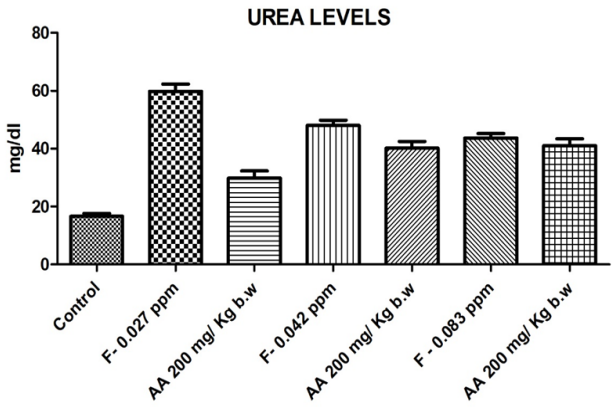

Fig. 1. *Effect of ascorbic acid (AA) on fenvalerate (F) induced toxicity showing serum urea levels in comparison to control. [Fenvalerate- 0.027 ppm, 0.042 ppm and 0.083 ppm respectively] (n=6, values are mean± S.D).*

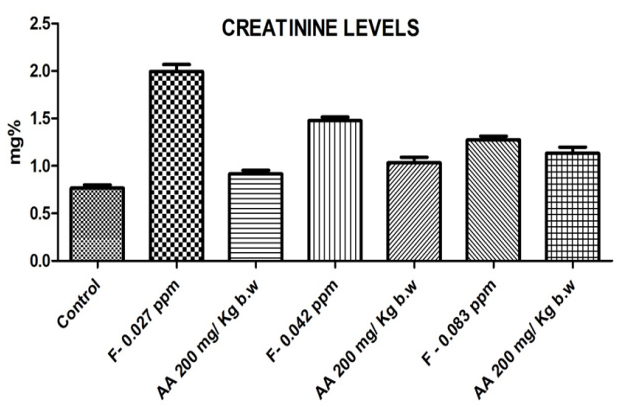

Fig. 2. *Effect of ascorbic acid (AA) on fenvalerate (F) induced toxicity showing serum creatinine levels in comparison to control. [Fenvalerate- 0.027 ppm, 0.042 ppm and 0.083 ppm respectively] (n=6, values are mean± S.D).*

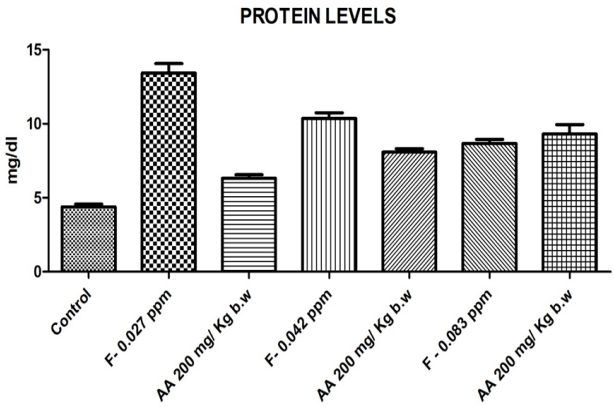

Fig. 3. *Effect of ascorbic acid (AA) on fenvalerate (F) induced toxicity showing serum protein levels in comparison to control. [Fenvalerate- 0.027 ppm, 0.042 ppm and 0.083 ppm respectively] (n=6, values are mean± S.D).*

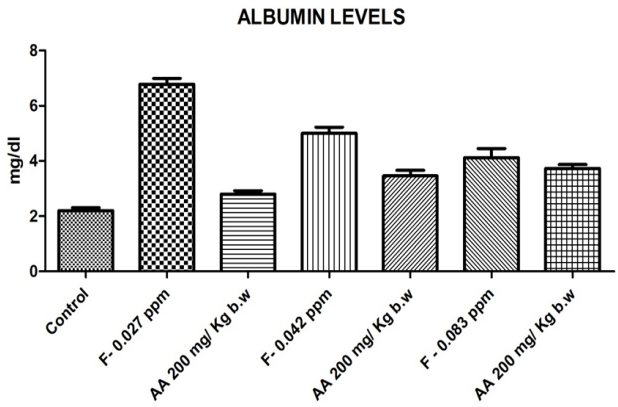

Fig. 4. *Effect of ascorbic acid (AA) on fenvalerate (F) induced toxicity showing Serum albumin levels in comparison to control. [Fenvalerate- 0.027 ppm, 0.042 ppm and 0.083 ppm respectively] (n=6, values are mean± S.D).*

10.37±0.378 with significant ($p<0.0001$) normalisation in ascorbic acid 200mg/kg b.w treated group was 8.083±0.2301, fenvalerate 0.083ppm treated was 8.667±0.272 with significant ($p<0.05$) normalisation in ascorbic acid 200mg/kg b.w treated was 9.30±0.642 (Fig. 3).

In serum albumin analysis, the level in control was 2.200±0.110 fenvalerate 0.027ppm treated was 6.775±0.217 with significant ($p<0.0001$) normalisation in ascorbic acid 200mg/kg b.w treated group was 2.800±0.125, fenvalerate 0.042ppm treated was 5.00±0.226 with significant ($p<0.0001$) normalisation in ascorbic acid 200mg/kg b.w treated group was 3.458±0.204, fenvalerate 0.083ppm treated was 4.117±0.331 with significant ($p<0.0001$) normalisation in ascorbic acid 200mg/kg b.w treated was 3.730±0.139 in (Fig. 4).

Histopathological evaluation: The control section of the kidney showed normal architecture of nephrocytes with Bowman's capsule and glomerulus. The convoluted and distal tubule architecture was quite normal denotes the normal physiological function of the nephrocytes (Fig. 5a. 1,2,3,4). In the Fenvalerate 0.027 ppm treated the section, showed abnormal architecture of nephrocytes with degeneration in the Bowman's capsule and glomerulus. The convoluted and distal tubules were also highly degenerated as the rupture in the epithelial membrane were clearly observed (Fig. 5b. 1,2,3,4). But after, ascorbic acid-treated upon 0.027 Fenvalerate exposed group, there was significant normalisation in the architecture of nephrocytes, glomerulus, Bowman's capsule, convoluted and distal tubules. (Fig. 5c. 1,2,3,4). In the Fenvalerate 0.042 ppm treated group, there was an observation of abnormal architecture of nephrocytes with a high degree of degeneration in the Bowman's capsule and glomerulus with the convoluted and distal tubules highly degenerated as the rupture in the epi-

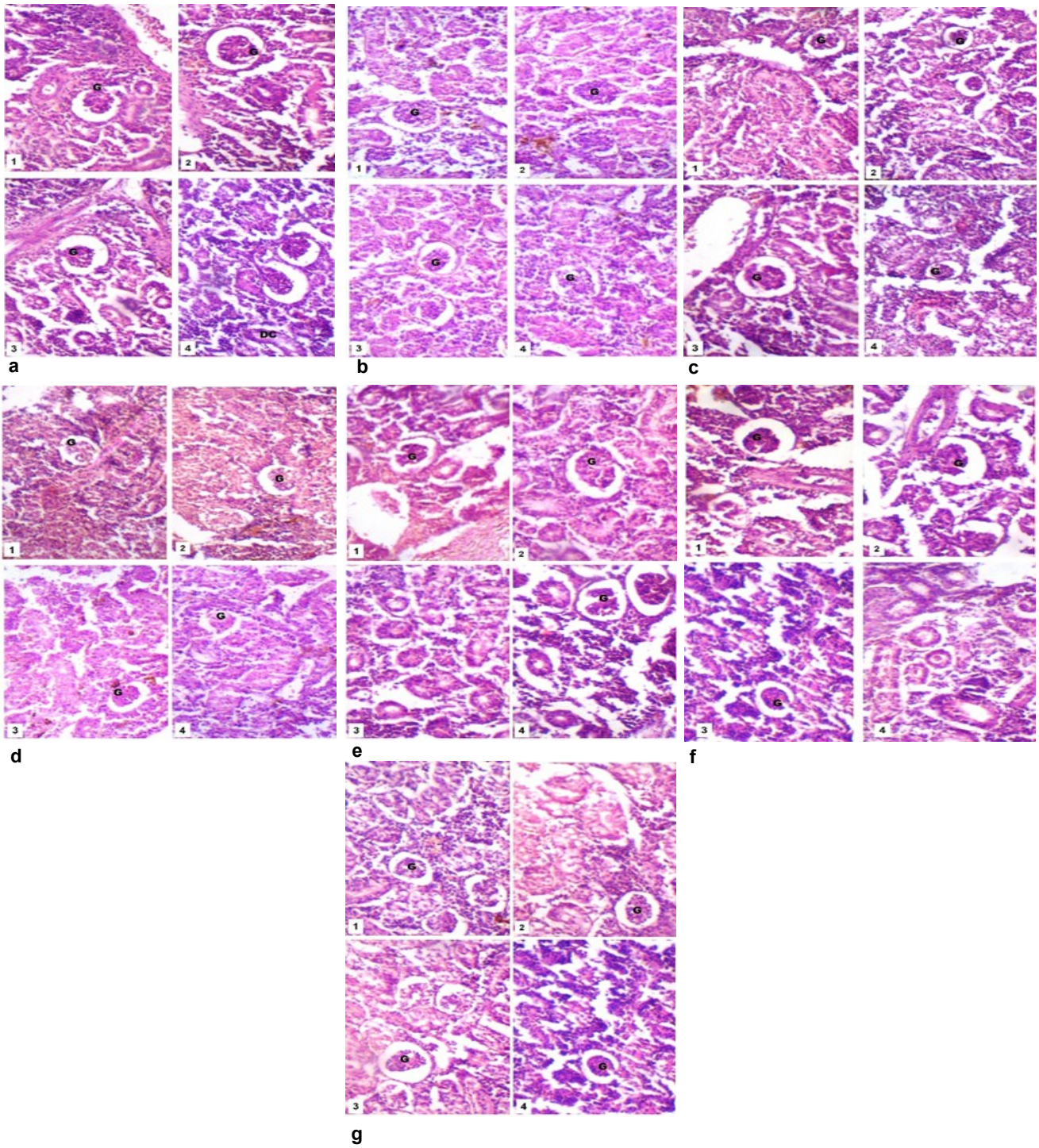

Fig. 5. *Section of kidney of C. batrachus: a (1 to 4) Control group showing normal architecture of nephrocytes with Bowman's capsule and glomerulus. x500. b (1 to 4) Fenvalerate 0.027 ppm showing the abnormal architecture of nephrocytes with degeneration in the Bowman's capsule and glomerulus along with the convoluted and distal tubules. x500. c (1 to 4) Ascorbic acid treated upon 0.027ppm fenvalerate exposed group there showing significant normalisation in the architecture of nephrocytes, glomerulus, Bowman's capsule, convoluted and distal tubules. x500. d (1 to 4) Fenvalerate 0.042 ppm showing high degree of degeneration in the Bowman's capsule and glomerulus with the convoluted and distal tubules highly degenerated as the rupture in the epithelial membrane can be seen. x500. e (1to4) Ascorbic acid treated upon 0.042 Fenvalerate exposed group showing significant normalisation in the nephrocytes especially in the Bowman's capsule and glomerulus. x500. e (1 to 4) Fenvalerate 0.083ppm treated group there is severe damage observed in the nephrocytes along with Bowman's capsule. x500. g (1 to 4) Ascorbic acid treated upon 0.083 Fenvalerate exposed group showing significant normalisation in the nephrocytes especially in the Bowman's capsule, glomerulus convoluted and distal tubules denotes the normal functioning of the kidney. x500.*

thelial membrane were clearly observed (Fig. 5d. 1,2,3,4). But after ascorbic acid treated upon 0.042 Fenvalerate exposed group showed significant normalisation in the nephrocytes especially in the Bowman's capsule and glomerulus. (Fig. 5e. 1,2,3,4). In Fenvalerate 0.083 ppm treated group there was severe damage observed in the nephrocytes along with Bowman's capsule and glomerulus especially the convoluted and distal tubules were highly degenerated as the rupture in the epithelial membrane were clearly observed (Fig. 5f. 1,2,3,4). But after Ascorbic acid treated upon 0.083 Fenvalerate exposed group there was significant normalisation in the nephrocytes especially in the Bowman's capsule, glomerulus convoluted and distal tubules denoted the normal function of the kidney (Fig. 5g. 1,2,3,4).

DISCUSSION

In recent times, due to excessive utilisation of pesticides for the better yield of crops has caused ill-effects to the aquatic ecosystem. The biomagnification of these pesticides through various food chains has reached the human food chain causing them various types of metabolic disorders as well as disease in them. The disease burden in the long duration of exposure is the cause of cancer as well (Katagi 2010; Hu *et al*., 2017; Clasen *et al*., 2018; Sabarwal *et al*., 2018; Mojiri *et al*., 2020; Yang *et al*., 2020). In the aquatic ecosystem, fishes are the best indicators of the toxicant exposure; hence the biochemical and histopathological parameters are the best to evaluate the pesticide toxicity (Sharma and Singh 2004 & 2006; Ramesh *et al*., 2014; Woo *et al*., 2018; Bojarski and Witeska 2020).

In the present study, the kidney function test of the fenvalerate exposed air-breathing fish *C. batrachus* showed that the exposure of fenvalerate on three different doses– 0.023ppm, 0.042ppm and 0.083ppm caused a severe deleterious effect on the biochemical parameters as there was significant ($p<0.0001$) rise in the urea (59.93±2.442, 48.0±1.844 and 43.67±1.542 respectively), creatinine ($p<0.0001$) (1.992±0.075, 1.475±0.038 and 1.275±0.038 respectively), protein ($p<0.05$) (13.43±0.639, 10.37±0.378 and 8.667±0.272 respectively) and albumin levels. (6.775±0.217, 5.00±0.226 and 4.117±0.331 respectively) But, after the administration of ascorbic acid there was significant ($p<0.0001$) normalisation in the levels as in the urea ($p<0.0001$) (29.83±2.469, 40.17±2.301 and 29.83±2.469 respectively), creatinine ($p<0.0001$) (0.916±0.035, 1.033±0.057 and 1.133±0.062 respectively), protein ($p<0.05$) (6.317±0.235, 8.083±0.2301 and 9.30±0.642 respectively) and albumin ($p<0.0001$) levels (2.800±0.125, 3.458±0.204 and 3.730±0.139 respectively).

Among the possible target organs, fenvalerate causes serious damage to the vital organs like liver and kidney as they are the most vulnerable part. Most of the toxicity is excreted by the kidney; hence, it exerts deleterious toxic effects through several mechanisms. Since urea is the end product of protein metabolism, the fenvalerate causes an increase in tubular permeability, leading to a decrease in the glomerular filtration rate. This causes decreased excretion and increased retention of nitrogenous wastes. The muscle contains the phosphocreatine, which undergoes spontaneous cyclization and forms creatinine which due to fenvalerate toxicity is severely affected and causes rise in the levels. Similarly, the proteins and albumin levels are also disrupted due to fenvalerate toxicity (Hohreiter *et al*., 1991 (*Oreochromis mossambicus*); Sun *et al*., 2007; Binukumari *et al*., 2016 (*Labeo rohita*); Brander *et al*., 2016 (vertebrates); Manavi *et al*., 2018; Vieira and Dos Reis Martinez, 2018 (*Prochilodus lineatus*); Velmurugan *et al*., 2018 (*Oreochromis mossambicus)*; Pico *et al*., 2019 (freshwater fish)). After the administration of ascorbic acid, there was a significant restoration in the biochemical levels denotes the antioxidant and antitoxic effect of it. Furthermore, ascorbic acid also controls the metabolic function through various pathways (Datta and Kaviraj 2003; Bhattacharya and Kaviraj 2009).

Besides, there was significant damage at the cellular level in different doses of fenvalerate exposed fish. The kidney tissue was severely damaged as the Bowman's capsule, glomerulus, the convoluted tubules. But, after the administration of ascorbic acid, there was a significant restoration in the kidney tissue. The antioxidant activity of ascorbic acid has caused restoration at the cellular level. Furthermore, the ascorbic acid also enhances the immune system of the fish, which helps to control the toxic effect of the pesticide. Various researchers have correlated the effect on other pesticides (chlorpyrifos) and heavy metals (cadmium) effect (Kumari and Sahoo 2005; Kumar *et al*. 2009; Narra *et al*., 2015; Narra 2017).

Hence, fenvalerate caused the deleterious effect to the fish at the biochemical level and histopathological level. Ascorbic acid plays a vital role to control the fenvalerate induced toxicity; hence, it possesses the nephroprotective effect.

Conclusion

The kidney function tests such as serum urea, creatinine, protein and albumin level of the fish increased due to fenvalerate induced toxicity. In addition to it, the normal architecture of histology of Bowman's capsule, convoluted tubules and distal tubules of kidney were disrupted due to intoxication of the pesticide in the fish. Ascorbic acid played a key role to combat the

fenvalerate induced nephrotoxicity in the freshwater fish *C. batrachus*. Hence it can be used to enhance the immune system of fish health, which will be beneficial to mankind.

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