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An Overview on the Impact of Vitamin C in Attenuating Toxic Effects of Fibronil in Nile Tilapia

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Abstract

A TWO-MONTH experiment was lined up to examine the harmful impacts of Fibronil in Nile Tilapia (*Oreochromis niloticus*) beside studying the mechanism by which vitamin C can attenuate these effects. One hundred eighty fish were randomly classified into four groups, with three replicates in each (n = 45/group, 15 per replicate). The first group kept as control, the second group was supplemented with vitamin C by (50 mg/L) in water, the third group was exposed to Fibronil by (0.0021 mg/L) (1/20 of 96 hr. LC50) in water, and the fourth group was exhibited to vitamin C and Fibronil together with the same dose of group two and three. There was a significant decrease in BWG and SGR in the fish of group three compared to control and group four. Total RBCs, Hb concentration, and Hct % of Fibronil treated group were significantly decreased than the other groups. The exposure to Fibronil results in a significant elevation of serum levels of ALP, ALT, AST, urea, creatinine, and cortisol with suppression of IgM and lysozyme activity. This toxic effect appeared to be improved by treatment with vitamin C in group (4). Supplementation of vitamin C led to higher Superoxide dismutase (SOD) levels and total antioxidant enzyme capacity. Liver, kidney and intestine of group (3) that treated with Fibronil revealed different histopathological changes which were improved by the addition of vitamin C in group four. Results showed improvement in performance, hematological, biochemical and antioxidant activities by addition of vitamin C.

Keywords: fibronil, hematology, immunity, histopathology, *Oreochromis niloticus*.

Introduction

Globally, freshwater ecosystems are significantly important but are currently threatened, experiencing a harsh decline in biodiversity [1]. Freshwater ecosystems face many threats: climate change, nutrient fluctuations, over-use, acidification, habitat destruction, and biological invasions. Furthermore, chemical pollution is a key issue, heavy use of pesticides in farming is the most typical form of this and pollutes waterways, endangering aquatic life [2].

Because of its advantageous traits, such as fast growth, ability to withstand poor environments, and the capacity to thrive on diverse protein diets, Nile tilapia (*Oreochromis niloticus*) is considered a highly valuable and commonly farmed fish globally [3].

Fibronil (FP), a wide-ranging phenylpyrazole insecticide, is officially known by the International Union of Pure and Applied Chemistry (IUPAC) as (±)-5-amino-1-(2, 6-dichloro-α, α, α-trifluoro-p-tolyl)-4-trifluoro methyl sulfinyl pyrazole-3-carbonitrile [4]. Moreover, the U.S. Environmental Protection Agency (US EPA) recognizes Fibronil, which is employed as a replacement for organophosphate chemicals [5]. Fibronil applied on many crops [6], as well as being used in aquaculture to manage the rice water weevil *(Lissorhoptrus oryzophilus Kuschel)* [7] and rice-crayfish issues *(Procambarus clarkii)* [8]. Fibronil usage has significantly increased, mainly replacing very dangerous and cancer-causing organochlorine and

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organophosphate insecticides [6]. Fibronil's metabolic byproducts impede GABAA-gated chloride channels in the central nervous system. This triggers excessive neuronal stimulation, which leads to insect death [9]. Fibronil poisoning in fish leads to injury of red blood cells [10], Immune system suppression, impairment of tissue's normal function and eventually death [11].

Because of the rise in the use of toxic substances in the environment, many studies are underway to identify solutions when animal organisms are exposed to insecticides, pesticides, and other harmful chemicals. Vitamin C, known scientifically as L-ascorbic acid, is regarded as an antioxidant, it is a strong reducing agent that prevents lipid peroxidation [12]. It's advantageous even in small quantities, for the redox regeneration of other antioxidants [13]. Vitamin C shields biological membranes from harm caused by peroxides. It acts by neutralizing singlet oxygen, superoxide, hydroxyl radicals, water-soluble peroxyl radicals, and hypochlorous acid. Consequently, it is recognized as a highly potent free radical scavenger in the fluids outside of cells [14]. Past research indicates that vitamin C can eliminate free radicals caused by pesticides, which lead to oxidative stress in fish [15]. Moreover, [16] indicated that vitamin C might serve as a feed supplement, enhancing the structure of the intestinal lining and boosting the immune response in tilapia.

Given that people eat a lot of Nile tilapia, and insecticides are used in farming, the harmful impact of these chemicals on fish tissue is really important to study. Thus, this research was done to assess how Fibronil exposure affects the well-being and immune system of *O. niloticus*. The research also aimed to see how vitamin C might reduce these effects. This was achieved by looking at blood-related, chemical, and immune system factors, along with examining the tissues under the microscope

Material and Methods

Ethical approval

This study was reviewed and approved by ZU-IACUC committee at Faculty of Veterinary Medicine, Zagazig University, Egypt. Ethical committee approval number: ZU-IACUC/2/F/261/2024

Chemicals

Fibronil (C12H4Cl2F6N4OS) (with a purity of 99.1%) was acquired from Bio Quest International Private Limited, located in Mumbai, India. The 96-hour LC50 value of FP for *O. niloticus* is 0.042 mg/l. [17].

Vitamin C (L-ascorbic acid), with the chemical formula C6H8O6, was acquired from Research-Lab Fine Chem Industries, located in Mumbai, India.

Fish collection and maintenance

We obtained Nile tilapia fingerlings (averaging 27.07 ± 0.15 g) (n=180) from Abbassa Fish Hatchery, Sharkia Province, Egypt. The fish were transported to the lab in plastic bags with ample hatchery water and oxygen. Upon arrival, the fish were put into well-aerated glass aquaria (dimensions: 80 × 40 × 30 cm) filled with 60 L of dechlorinated tap water, with around 25% of the water changed daily. For two weeks before the experiment began, the fish were kept in the aquaria and given a basal diet (Table 1). Water quality parameters were computed following [18]. Throughout the experiment, dissolved oxygen (7.17 ± 0.4 mg L–1), temperature (22.4 ± 1.02 ℃), pH (6.7 ± 0.1), ammonia-N (0.035 ± 0.01 mg L–1), and nitrite levels (0.12 ± 0.02 mg L–1), along with a set photoperiod (12 h light, 12 h dark) were kept up in the lab. Waste products were removed each day by siphoning to avert the stress of ammonia buildup. The water parameters were checked twice daily throughout the study to keep the values of parameters inside the range that's recommended. To avoid a decline in water quality, any dead fish were promptly taken away, and the tanks were refilled with clean water.

Experimental protocol and diet preparation

Fish were classified randomly into four groups with three replicates in each (n=45/group, 15/replicate), group one (G1) kept as normal control, group two (G2) was supplemented with vit. C by (50 mg/L) [19] in water for 2 months, group three (G3) was subjected to Fibronil by (0.0021 mg/L) (1/20 of 96 hr. LC50) [20] in water for 2 months and group four (G4) was subjected to both vit. C and Fibronil by 50 mg/L and 0.0021 mg/L respectively for 2 months.

The fish were given 5% Bwt. of their specific diets, three times daily. The diets were formulated at the Fish Research Center, which is part of the Faculty of Veterinary Medicine, Zagazig University, Egypt. The baseline diet, in dry pellet form, consisted of 2944.41 kcal/kg metabolizable energy (ME) and 30.80% CP and was intended to fulfill the nutritional needs of *O. niloticus* [21] (Table 1).

Sampling

At the end of the experiment after 24 hours of fasting, fish were chosen and anesthetized using buffered tricane-methane sulfonate (E10521-10G; Sigma–Aldrich) (100 mg/L; for 5 min.) [22]. Blood samples were collected from caudal vein in two syringe one with anticoagulant for hematological studies and the other one without anticoagulant for serum separation, serum was stored at -20 ℃ until use for biochemical and immunological studies.

Growth performance and mortality rate

Initial and final body weight and feed consumption were recorded. Feed conversion ratio (FCR), percentage of body weight gain (BWG %), and specific growth rate (SGR %) were computed based on the data [23] by the following equation:

- Body weight gain (g/fish) = (WT–WI) [where WT = final weight of the fish in grams and WI = initial fish weight in grams]
- FCR = total feed intake (g)/body weight gain (g)
- SGR (%/day) = 100 × (ln WT–ln)/time in days [where ln denotes natural logarithm]
- Survival rate (%) = (final fish count in each group / initial fish count) × 100.

Hematological parameters investigation

We determined erythrocyte counts using an improved Neubauer hemocytometer, employing Natt and Herrick solution as a specific diluent, as described by [24]. The packed cell volume was determined using a micro hematocrit centrifuge, as mentioned by [25]. Hemoglobin concentration was determined via the cyanmethemoglobin colorimetric technique following centrifugation, as earlier expressed by [26]. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated. Blood smears were made, set with methyl alcohol, and colored using Giemsa to assess the differential white blood cell count, as described by [27].

Biochemical parameters investigation

All biochemical parameters were measured using commercially available kits, each parameter followed the manufacturer's manual's recommended technique. The liver transferases (alanine aminotransferase ALT and aspartate aminotransferase AST) activities were estimated according to [28]. ALP was estimated according to [29]. According to [30], serum urea was measured, and serum creatinine was approximated using [31], Serum lysozyme activity was determined according to [32].

Serum levels of cortisol were detected using the ELISA technique and test kits supplied by CUSABIO, Catalog Number, and CSB-E05112r. Determination of immunoglobulin M (IgM) was according to [33].

Antioxidant capacity

Malondialdehyde concentrations (MDA) were measured using the protocol outlined by [34], superoxide dismutase (SOD) was calculated using a colorimeter according to [35]. Total antioxidant capacity (TAC), assessed spectrophotometrically using commercially available kits (Bio-Diagnostic Company, Cairo, Egypt; catalog number TA2513).

Histopathological examination

Samples from the liver, kidney, and gut were collected. They were preserved in a 10% buffered neutral formalin solution, dehydrated using a series of ethanol concentrations (70-100%), made transparent with xylene, and then embedded in paraffin wax. Afterward, the samples were stained with Hematoxylin and Eosin (H&E) stains and examined under a microscope [36].

Statistical analysis

Analysis of variance (ANOVA) was used for the statistical analysis. Duncan's Multiple Range test [37] identified treatment differences, at a 0.05 significance level. The SPSS software [38] was employed for all statistical evaluations.

Results

Growth performance

The detailed growth performance parameters are listed in (Table 2) as, BWG and SGR were significantly higher in the vitamin C treated group (G2) than the control group (G1) ($p<0.05$), in contrary the previous parameters were significantly lower than control in Fibronil treated group (G3) and the group treated with both Fibronil and vitamin C (G4) ($p<0.05$). FI and FCR were increased significantly in Fibronil treated group than in other groups, on the other hand, the same parameters were significantly decreased ($p<0.05$) in vitamin C treated group than in other groups. The highest survival rate was recorded in group (G2) which was supplemented with vitamin C, also, group (G4) recorded an increased survival rate than group (G3).

Hematological evaluation

Our results as, noted in (Table 3) revealed significant decrease ($p<0.05$) in each of the total erythrocytic counts (RBCs), hemoglobin concentrations (Hb) and hematocrit (Hct) of Fibronil treated group than the other groups, additionally it showed significantly higher mean corpuscular volume (MCV) ($p<0.05$). Group 4 (treated with both Fibronil and vitamin C) showed non-significant changes in erythrogram than the control group.

Concerning leukogram, (G2 & G4) that were treated with vitamin C announced a significant release ($p<0.05$) in total leukocytic, lymphocytic, and monocytic counts than control and Fibronil-treated-groups while Fibronil treated group showed decreased lymphocytic count than the other groups.

Serum biochemical indices

The impact of Fibronil, vitamin C, and both of them on serum biochemical parameters and antioxidant enzyme capacity were documented in tables (4) and (5) respectively. The serum activities of AST, ALT, ALP, urea, creatinine and cortisol showed a significant increase in (G3) when

compared with (G1). Group 4 which exposed to vitamin C and Fibronil showed a significant increase in ALP, urea, and cortisol levels compared with (G1) while it showed non-significant increase in ALT, AST and creatinine. Group 2 which received vitamin C showed non-significant increase in ALP, AST and urea when compared with control group. A significant decrease recorded in ALT and cortisol with a significant increase in creatinine in (G2) when compared with (G1).

Serum immunological and antioxidant activities

Group 2 (G2) and (G4) showed significant improvement in IgM, lysozyme activity and MDA compared with (G3), which revealed a significant decrease in IgM and lysozyme activity as well as a significant increase in MDA when compared with (G1).

Superoxide dismutase (SOD) showed a significant increase in (G2) while it showed a non-significant change in G3 and G4 when compared with (G1). Total antioxidant enzyme capacity showed a significant increase in (G2) and a significant decrease in G3 and G4 compared to control group.

Histopathological analysis

The liver in (G3) revealed congestion of the hepatic blood vessels, pyknosis, karyolysis, and coagulative necrosis in the hepatocytes and pancreatic islands. Moderate inflammatory cell infiltration around the pancreatic island (Fig. 1a). Some instances (Fig. 1b) showed diffuse fatty changes, vacuolations and hydropic degenerations in the hepatocytes. While, the hepatopancreas of (G4) displayed notable betterment with remaining slight vacuolar deterioration in the liver cells (Fig.1c). The pancreatic islands showed mild congestion focal hepatic cellular degeneration, focal areas of hepatic necrosis, peri-portal aggregations of melano-macrophages and round cell infiltration.

Regarding (Fig. 2a), the kidney of (G3) exhibited severe necrosis of tubular cells, with congestion, degeneration glomeruli, enlarged Bowman's space, edema, atrophic cells, and shrinkage of the lumen of renal tubules in (Fig. 2b). In contrast, (Fig. 2c) exhibited the kidney of (G4) which revealed some preservation of histological structure, but some cloudy swelling of the renal tubules, congested blood vessels and shrinkage of some glomeruli were present.

The intestine of (G3) showed severe necrosis of the villi, complete sloughing, infiltrated by lymphocytes and macrophages, and desquamated epithelium of intestinal lamina (Fig. 3a). The intestine of (G4) showed focal necrotic villi, mucinous degeneration, goblet cell, and infiltrated by leucocytes in the lamina propria (Fig. 3b).

Discussion

Water-soluble vitamin C has been documented to remain stable when dissolved in water [39]. In our study we investigated the effect of addition of vitamin C directly into aquaria water to attenuate the biochemical and tissue damage after exposure of Tilapia fish to toxic dose of Fibronil.

As outlined in results table (2) indicating that vitamin C supplementation resulted in improved performance of Nile tilapia fish even with exposure to Fibronil. This might be linked to vitamin C's role in boosting growth hormone in the bloodstream, modifying the intestine's form, and fortifying the gut's surface for absorption in fish [16]. Vitamin C ability to energize protein synthesis may explain why fish grow faster and gain weight [40, 41], additionally, vitamin c accelerating the digestibility and absorption of nutrients in fish′ s body as explained by [42] Our results revealed increased FBW and WG with decreased FI and FCR in groups treated with vitamin C and this was in harmony with [43, 44] in Nile tilapia *(Oreochromis niloticus)* and in common carp *(Cyprinus carpio)* [445]. Also, we are in agreement with Nile tilapia supplemented with dietary vitamin C and vitamin E by a level 420 and 100 mg/kg achieving high growth performance and feed utilization compared to the control [46].while, [47] were in difference with us as they found that the parameters of juvenile large yellow croaker were unaffected by dietary vitamin C. Fish survival improved after being treated with vitamin C. This supports earlier research suggesting that vitamin C can lessen the effects of toxins and boost fish survival by helping to return physiological functions to normal [48].

Concerning hematological indices, we concluded that Fibronil leads to decreased RBCs counts, Hb conc., Hct % and lymphocytic count. Previously, [10] discussed that a Fipronil concentration of 0.0002 mg/L (0.2 µg/L) leads to damage in the red blood cells of silver catfish, *Rhamdia quelen,* resulting from the harmful effects of Fibronil on the creation of red blood cells. A lowered Hb level could result from the quick conversion of hemoglobin to methemoglobin. Alternatively, it might be due to the release of oxygen radicals, a consequence of the toxic impact and oxidative stress caused by Fibronil, as found by [49]. Scaling down of hematological values could be also due to a substantial reduction of hematopoiesis in kidneys. Moreover, we found that these effects can be counteracted by the addition of vitamin C as vitamin C functions not only as a free radical scavenger [48], but also elevates fish's innate immunity [50].

The findings mirrored those of [51] as they found that exposing carp fry *(Caprinus carpio)* to a sub lethal dose (1/10th LC50) of Fibronil for 45 days led to a considerable reduction in red blood

cell count, total white blood cell count (WBC), and hemoglobin percentage. Nevertheless, these findings were at odds with those reported by [52] who reported increased erythrocyte, pack cell volume, and platelets in *Clarias gariepinus* exposed to pesticide. Increased heterophil count in Fibronil-exposed groups may be attributed to immunological reactions expressive of injury to different tissues in exposed fish [53] and this was similar to results reported by [54] in common carp, *Cyprinus carpio*, also, with [46] who reported increased WBCs count in Nile Tilapia supplemented with vitamin C and vitamin E in their diet.

In terms of serum biochemistry and immunology, the elevation in liver enzymes (ALP, ALT, and AST) and cortisol in the Fibronil-exposed group indicated Fibronil's harmful impacts on the liver and overall health. ALT serves as a marker for liver health and is highly susceptible to pollutant toxicity [55]. The increasing which observed in the liver AST is attributed to mitochondrial membrane damage [56], the same outcome was explained by [57] who imputed this increase to either enzyme induction from insecticide-induced stress or the detrimental influence of the insecticide on oxidation via the Kreb's cycle. Consequently, the notable rises in liver AST and ALT observed in this research might be linked to the stress response caused by Fibronil (used as an insecticide) and its potential for liver toxicity.

Supplementation with vitamin C reduces the harmful effect of Fibronil, and this effect was seen through the improvements in serum ALP, ALT, AST, urea and cortisol levels in group which treated with both vitamin C and Fibronil. These results were in agreement with [58] who reported that, the ALT and AST levels exhibited significant decrease after treatment with adequate vitamin C, suggesting the efficacy of this vitamin in protecting hepatic cells from damage. Also, ourfinding was in harmony with [59] who reported that optimal dietary vitamin C is helpful to keep the normal function of the liver in juvenile Chinese sucker (*Myxo cyprinus asiaticus*) and juvenile striped catfish (*Pangasianodon hypophthalmus*).

Creatinine is filtered out by kidney thus; blood creatinine level can interpret how well the kidneys are working [60]. Our study showed that the Fibronil-treated group had elevated levels of urea and creatinine that result in the same line as [17] who reported that this elevation could be attributed to Fibronil's impact on the kidney's detoxification function. Elevated cortisol level was noted and this was in accordance with [51] who stated that cortisol level in *Cyprinus carpio* fry were seen to increase after a 45-day exposure to Fibronil at 0.0428 mg/l (equal to one-tenth of the 96-hour LC50).

Both IgM and lysozyme are helpful indicators of immune health in fish. Vitamin C is known to improve immune function as it boosting macrophage presence, complement system function, lysozyme amounts, leucocyte phagocytosis, cytokine synthesis, and antibody production [16]. Our experiment revealed that Fibronil exposure reduced the serum levels of IgM and lysozyme activity when compared to a control. Conversely, using vitamin C either alone or together with Fibronil was effectively improved their levels, This improvement may correlate to the stabilization of gut microbiota, physiological adaptation and accelerated immunological responses as explained by [61,62]. These findings were in agreement with the results of [63] on *Cyprinus carpio*.

Antioxidants like vitamin C are responsible for protecting cells from harms caused by neutralizing free radicals [64]. Increasing of MDA and decreasing in TAC in the Fibronil exposed group were observed in this study, like us [49] observed that exposing *Cyprinus carpio* to 0.65 mg/l of Fibronil for 7, 30, and 90 days yielded sever harmful consequences expressed by clear decreases in the quantities of antioxidant enzymes and immune function. Like us [65] stated elevated levels of MDA linked to exposure to Fibronil and fibronil sulfone. These effects may be related to the ability of Fibronil to raise lipid peroxidation (suggested by MDA levels) and suppress GSH level, in addition to its suppressive effect on the expression of hepatic antioxidant enzymes CAT and SOD. In this study, the fish consuming a vitamin C treated diet showed increased serum SOD levels and overall antioxidant capacity. There is an improvement in the results of SOD and MAD in the group treated with both vitamin C and Fibronil when compared with the Fibronil group. These findings were linked to the potent antioxidant properties of vitamin C, as documented earlier by [66]. This also restores typical cell functions related to cell survival, inflammatory responses and cells' capacity for phagocytosis.The improvement of antioxidant enzymes may related to vitamin c's ability to donate electrons as mentioned by [67]. Our results also corresponded to those that presented by [68] for Juvenile yellow catfish *(Pelteobagrus fulvidraco)* that were fed a diet with an added 156.5 mg kg–1 of vitamin C, and those by [69] for juvenile for young black carp *(Mylopharyngodon piceus)* that were fed a diet with 63.0 mg kg–1 vitamin C added. Our results also, similar to [42] who reported elevated TAC, SOD, CAT, and GSH-Px with decreased levels of MDA in the serum of Nile tilapia that supplemented with vitamin c.

For histopathological alterations liver of Fibronil subjected group (G3) exhibited congestion of hepatic blood vessels with necrosis, fatty changes, vacuolation and hydropic degeneration in hepatocytes, these findings go in harmony with [54] who recorded congestion, necrosis of hepatocytes, increased sinusoidal space and fatty infiltration in the liver of common carp that received different

concentrations of Fibronil and also, in accordance with previous studies of [70] which were done on fish exposed to different kind of pollutants, this confirms a general toxic effect of Fibronil on Nile Tilapia liver. In this study supplementation of vitamin C in G4 led to modulation of toxic effect of Fibronil presented by presence of melano-macrophage which indicate increasing the fish immunity, in a similar vein [43] said that liver sections from Nile tilapia that supplemented with 200 mg/kg-1 diet of vitamin C exhibited normal hepatic histo-structures with congested sinusoids and fish supplemented with 300 mg/kg–1 diet of vitamin C presented normal hepatic tissues with just a few Kupfer cell hyperplasia while fish fed 400 mg/kg-1 diet showed normal liver architecture with few lipid vacuoles which may due to the antioxidant effect of vitamin C's hepatoprotective effect [71].

Fish kidneys are crucial for removing waste, producing red blood cells, and maintaining balance in their watery habitat [72]. Our research on Nile Tilapia kidneys exposed to Fibronil showed substantial necrosis of tubular cells, congestion, glomeruli degeneration, expanded Bowman's space, edema, and atrophic tubular epithelium cells, comparable to findings by [54] in common carp supplemented with Fibronil.

Regarding the intestine of (G3), our finding showed severe necrosis of the villi, lymphocytes, and macrophage infiltration in the submucosa and desquamated epithelium of intestinal Lamina. These findings were in harmony with the findings of [17] who supplemented Nile tilapia with different concentrations of Fibronil.

The improvement of intestinal histology in G4 may be due to the effect of vitamin C in attenuating the toxic effect of Fibronil and this was in the same *line with [16] who mentioned that Nile tilapia fed a* vitamin-C-treated diet for 28 days had better intestinal anatomy. Also, in agreement with [43] who reported that dietary vitamin C improved villus height, goblet cell count, and IELs. This confirms boosted intestinal absorption and immune defense, and as a result, growth efficiency. In the contrary, [73] recorded that no pathological changes take place in the intestine of rainbow trout fed oxidized fish oil in conjugation with of vitamin C and vitamin E.

Conclusion

It can be concluded that extended exposure of Nile tilapia (*O. niloticus*) to Fibronil led to stunted growth, blood and biochemical shifts, weakened immunity, and oxidative stress. Furthermore, employing vitamin C as a natural feed supplement was seen as a valuable dietary addition for its protective effects against the detrimental effects of Fibronil. Recommendations for consistent supplementation of vitamin C in aqua feed are important for boosting the overall well-being of fish, helping them to combat serious toxicological effects of certain newly-appearing aquatic pollutants.

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Declaration of Conflict of Interest

No Conflict of interest

TABLE 1. Ingredients and calculated composition of the basal diet.

Ingredient%	
10	Wheat flour
35	Yellow corn
18	Soyabean meal
16	Fish meal
14	Poultry byproduct meal
5.5	Vegetable oil
1.5	Vitamin and mineral mixtures[a]
Calculated composition%	
84.28	Dry matter
30.79	Crude protein
9.92	Ether extract
2.40	Crude fiber
7.09	Ash
38.99	Nitrogen-free extract
2944.41	Digestible energy[b]

a. Vitamin and mineral supplement (alfakema): Each 1 kg provided 580000 IU vit. A, 8600 IU vit. D3, 0.1 mg vit. C, 720 mg vit. E, 142 mg vit. K3, 58 mg, vit. B1, 34 mg vit. B2, 34 mg vit. B6, 58 mg vit. B12, 8 mg pantothenic acid, 86 mg folic acid, 65 mg manganese sulfate, 3000 mg zinc methionine, 2000 mg iron sulfate, 3400 mg copper sulfate, 25 mg calcium iodide, 572 mg cobalt sulfate, 25 mg sodium selenite, , and calcium z (filler) to 1000 g.

b. Calculating digestible energy using the values: 3.5 kcal/g for protein, 8.1 kcal/g for fat, and 2.5 kcal/g for nitrogen-free extract**.**

TABLE 2. Impacts of treatment with Fibronil and vitamin C on growth performance of Nile tilapia.

Groups parameters	Group 1	Group 2	Group 3	Group 4
IBW (g)	27.04±0.17	27.02±0.13	27.18±0.11	27.06±0.15
FBW (g)	53.46±0.23^{b}	62.16±0.31^{a}	46.2±0.19^{d}	51.4±0.29^{c}
BWG (g)	26.42±0.12^{b}	35.14±0.18^{a}	19.03±0.08^{d}	24.34±0.14^{c}
SGR (%/day)	1.13±0.01^{b}	1.38±0.00^{a}	0.88±0.00^{d}	1.07±0.002^{c}
FI (g/fish)	39.76±0.16^{b}	38.64±0.13^{c}	40.92±0.16^{a}	40.08±0.10^{b}
FCR	1.5±.01^{c}	1.1±.01^{d}	2.15±.01^{a}	1.65±.01^{b}
Survival %	94.7	97.4	71	77

The data is presented as the mean ± standard error. Values with different superscript letters in the same row show a significant difference (P<0.05). (n=5).

TABLE 3. Impacts of treatment with Fibronil and vitamin C on hemogram of Nile tilapia:

Groups parameters	Group 1	Group 2	Group 3	Group 4
RBCs (X 10^6/cmm	3.32 ± 0.18 a	3.74 ± 0.24 a	2.78 ± 0.13 b	3.28 ± 0.16 a
Hb (g/dl)	11.66 ± 0.34 a	12.06 ± 0.38 a	10.06 ± 0.21 b	11.16 ± 0.29 a
PCV (%)	34.34 ± 0.63 b	37.2 ± 0.88 a	31.66 ± 0.42 c	33.12 ± 0.58 bc
MCV (fl)	103.43 ± 1.80 b	99.47 ± 1.70 b	113.88 ± 2.90 a	100.98 ± 2.30 b
MCH (pg)	35.12 ± 1.18	32.25 ± 1.32	36.18 ± 1.42	34.02 ± 1.25
MCHC (g/dl)	33.95 ± 1.15	32.42 ± 1.09	31.17 ± 1.18	33.69 ± 1.24
WBCs (X10^3/cmm)	39.16 ± 1.82 b	46.96 ± 1.16 a	40.34 ± 0.96 b	44.67 ± 1.27 a
Lymphocytes	26.16 ± 0.91 b	32.29 ± 0.93 a	22.36 ± 0.84 c	28.08 ± 0.88 b
Heterophils	8.88 ± 0.71 b	9.79 ± 0.63 b	13.98 ± 0.82 a	12.16 ± 0.74 a
Monocytes	1.89 ± 0.28 ab	2.54 ± 0.40 a	1.61 ± 0.23 b	2.12 ± 0.36 ab
Eosinophils	1.42 ± 0.19	1.51 ± 0.21	1.54 ± 0.23	1.49 ± 0.18
Basophils	0.81 ± 0.09	0.83 ± 0.08	0.85 ± 0.07	0.82 ± 0.08

The data is presented as the mean ± standard error. Values with different superscript letters in the same row show a significant difference (P<0.05). (n=5).

TABLE 4. Impacts of treatment with Fibronil and vitamin C on serum biochemical parameters of Nile tilapia.

Groups parameters	Group 1	Group 2	Group 3	Group 4
P (µg/ml)	162.60±6.43^{c}	149.00±7.92^{c}	242.20±21.03^{a}	203.80±9.39^{b}
T (µg/ml)	41.60±2.06^{b}	28.80±2.09^{c}	69.30±2.49^{a}	43.70±1.91^{b}
Γ (µg/ml)	103.20±3.09^{b}	73.80±10.07^{b}	179.40±17.30^{a}	86.40±8.35^{b}
a (mg/dl)	33.84±1.06^{c}	34.66±1.77^{c}	55.36±3.32^{a}	42.64±0.77^{b}
atinine (mg/dl)	0.32±0.02^{b}	0.55±0.04^{a}	0.60±0.05^{a}	0.42±0.04^{b}
tisol (ng/ml)	9.74±0.07^{c}	9.07±0.25^{d}	12.95±0.17^{a}	10.24±0.06^{b}

The data is presented as the mean ± standard error. Values with different superscript letters in the same row show a significant difference (P<0.05). (n=5).

TABLE 5. Impacts of Fibronil and vitamin C on some serum immunological and antioxidant activities of Nile Tilapia.

Groups parameters	Group 1	Group 2	Group 3	Group 4
IgM (µg/ml)	4.10±0.29^{a}	4.55±0.42^{a}	2.80±0.12^{b}	4.50±0.17^{a}
Lysozyme activity (Unit/ml)	41.80±2.83^{a}	51.20±3.73^{a}	17.40±3.70^{b}	44.40±1.86^{a}
MDA (nmol/L)	106.40±8.69^{b}	102.60±5.24^{b}	141.40±10.11^{a}	110.00±3.50^{b}
SOD (U/L)	43.80±7.48^{b}	60.20±3.99^{a}	42.20±2.48^{b}	51.20±2.99$^{a\ b}$
TAC (µmol/L)	432.60±13.20^{b}	546.00±14.97^{a}	351.60±9.55^{c}	368.40±8.35^{c}

The data is presented as the mean ± standard error. Values with different superscript letters in the same row show a significant difference (P<0.05). (n=5).

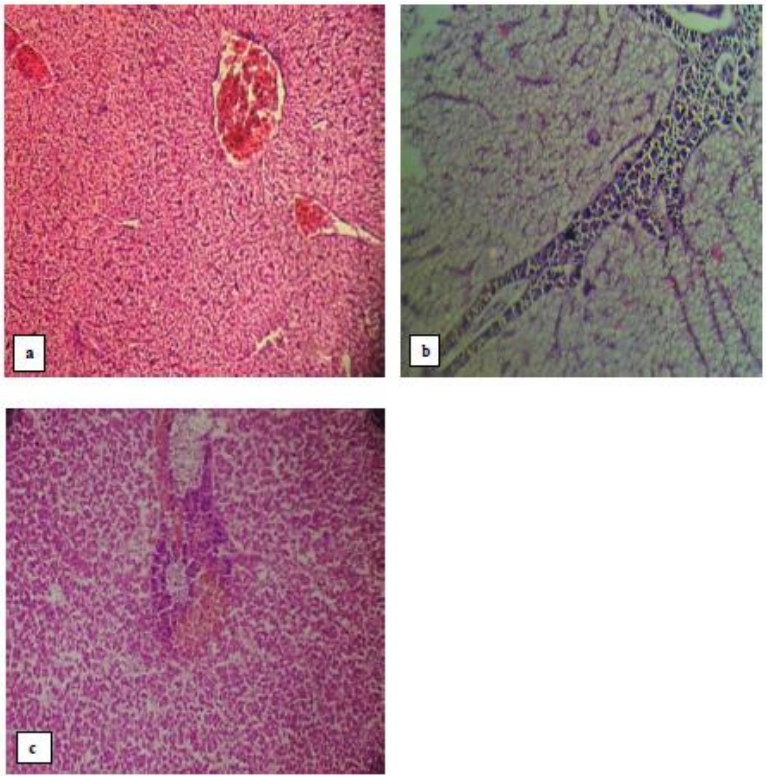

Fig. 1a. Section of liver of group (3) showing focal hepatic necrosis, thrombus in portal vein and hepatic sinusoid (H&E X200), Fig. 1b: Section of liver of group (3) showing diffuse fatty changes, vacuolations, and hydropic degenerations in the hepatocytes (H&E X200), Fig. 1c: Section of liver of group (4) showing focal areas of hepatic necrosis, and peri-portal aggregations of melano–macrophages (H&E X200).

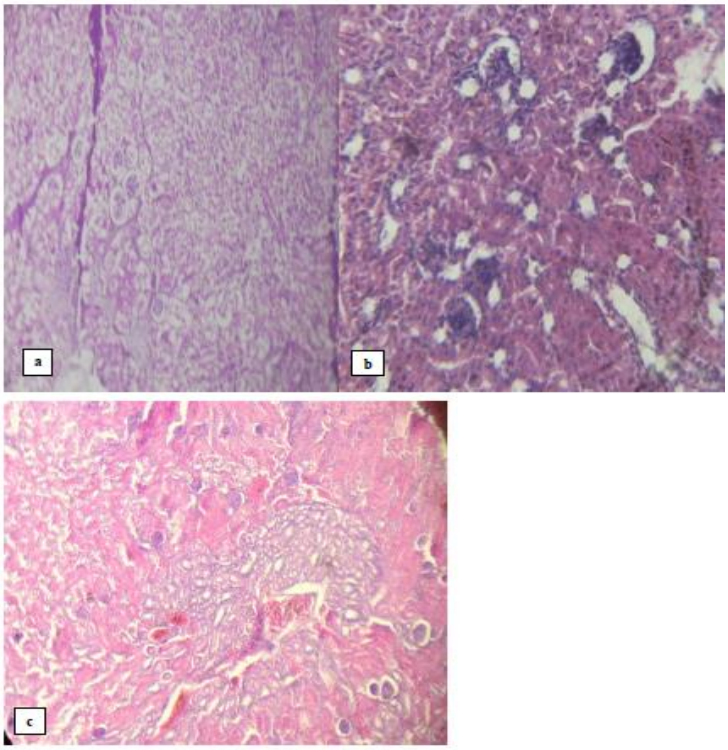

Fig. 2a. Section of kidney of group (3) showing severe necrosis of the renal tubular cells (H&E X100), Fig. 2b: Section of kidney of group (3) showing degeneration of glomeruli, increased Bowman's space, edema, atrophic cells, and atrophy of the lumen of renal tubules (H&E X200), Fig. 2c: Section of kidney of group (4) showing cloudy swelling of the renal tubules, congested blood vessels and shrinkage of some glomeruli (H&E X100).

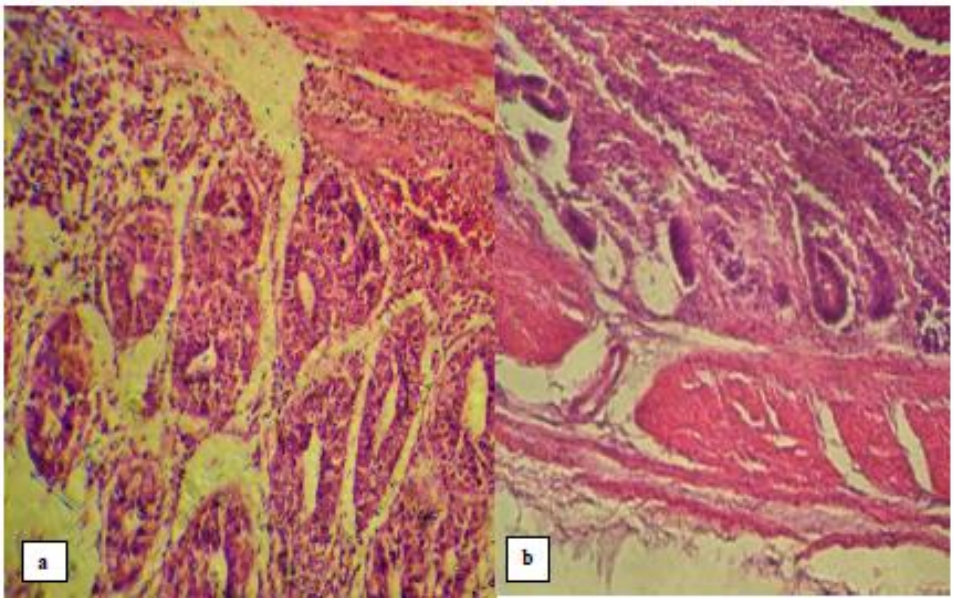

Fig. 3a. Section of intestine of group (3) showing severe necrosis of the villi, complete sloughing, submucosa infiltrated by lymphocytes and macrophages (H&E X200), Fig. 3b: Section of intestine of group (4) showing focal necrotic villi, mucinous degeneration, goblet cell, and infiltration by leucocytes in the lamina propria (H&E X200).

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نظرة عامة حول تأثير فيتامين سي في معادلة التأثيرات السامة للفبرونيل في أسماك البلطي النيلي

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الملخص

أعدت هذه التجربة لفحص التأثيرات الضارة للفيبرونيل في أسماك البلطي النيلي ودراسة الآلية التي يقوم من خلالها فيتامين سى بمعادلة هذه التأثيرات وذلك من خلال تقييم المعايير الدموية والكيميائية الحيوية والمناعية بالإضافة إلى الفحص النسيجي المرضي. تم توزيع مائة وثمانون سمكة عشوائيًا إلى أربع مجموعات، بثلاث تكرارات في كل مجموعة (عدد الأسماك = ٤٥ سمكة/مجموعة، ١٥ سمكة لكل تكرار). حُفظت المجموعة الأولى كمجموعة ضابطة، بينما أضيف فيتامين سى الى المجموعة الثانية فى المياه بنسبة (٥٠ ملجم/لتر) لمدة شهرين، بينما عُرّضت المجموعة الثالثة للفبرونيل (٠,٠٠٢١ ملجم/لتر) فى المياه لمدة شهرين وعُرِّضت المجموعة الرابعة لفيتامين سى والفبرونيل معًا بنفس الجرعة و لنفس المدة كما في المجموعتين الثانية والثالثة. سجلت التجربة انخفاض كبير في أكتساب الوزن و معدل النمو الخاص في أسماك المجموعة الثالثة مقارنةً بالمجموعة الضابطة والمجموعة الرابعة. انخفض إجمالي خلايا الدم الحمراء وتركيز الهيموجلوبين و حجم الخلايا المضغوطة في المجموعة المعالجة بالفيبرونيل بشكل كبير مقارنةً بالمجموعات الأخرى. أدى التعرض للفيبرونيل إلى ارتفاع كبير في مستويات السيروم من الألكلاين فوسفاتيز و الألانين أمينوترانسفيريز و الأسبارتات أمينوترانسفيريز واليوريا والكرياتينين والكورتيزول مع تثبيط نشاط االجلوبيولين المناعى ام والليسوزيم، ويبدو أن هذا التأثير السام قد تحسن بالعلاج بفيتامين سى في المجموعة الرابعة. أدت المعالجة بفيتامين سى إلى ارتفاع مستويات سوبر أوكسيد ديسميوتيز والقدرة الكلية لإنزيمات مضادات الأكسدة. أظهر الكبد والكلى والأمعاء في المجموعات المعالجة بالفيبرونيل تغيرات نسيجية مرضية مختلفة والتي تحسنت بإضافة فيتامين سى في المجموعة الرابعة. أظهرت مجموعة الأسماك التي تم تزويدها بفيتامين سي بالإضافة إلى الفيبرونيل تحسنًا في الأداء والأنشطة الدموية والكيميائية الحيوية ومضادات الأكسدة مقارنة بالمجموعة المعالجة بالفيبرونيل، وبالتالي فإن فيتامين سي هو مكمل مناسب للتخفيف من التأثير السام للفيبرونيل.

الكلمات الدالة: فيبرونيل ، أمراض الدم ، المناعة ، التشريح المرضى ، البلطى النيلى.