Effects of Acute Fenitrothion Insecticide Exposure on DNA Damage and Oxidative Stress Biomarkers and Health of Nile Tilapia Fingerlings, *Oreochromis niloticus* L

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**Abstract:** A wide range of production and application of pesticides is continues to rise and accompanied with serious problems of health hazards. The objective of the present study isto assess the injurious effect of one of organophosphate insecticides fenitrothion (FNT) on Nile tilapia, *Oreochromis niloticus* L. fingerlings via determination of its 96 h median lethal concentration (LC₅₀), behavior changes, DNA damaging potential using SCGE assay in gills, pro-oxidant activity via detecting serum reduced glutathione content (GSH), superoxide dismutase enzyme activity (SOD) and malondialdehyde (MDA) concentration. Biochemical estimation of serum ALT, cortisol, creatinine, urea and level of IgM.Histological alterations occurred in liver, kidneys and gills following acute low and high sublethal FNT exposure. In this study 240 *O. niloticus* L. fingerlings were used. Mortality was investigated in FNT exposed fishes during 96 h to concentrations ranging from zero to 7mg/ L. The estimated 96 h LC₅₀ was 4.7 mg/L. Significant increase in tail length, tail DNA % and TM in FNT-exposed groups. Significant reduction in serum GSH, increase in SOD, MDA, ALT, cortisol, creatinine and urea levels in groups of FNT-exposed fish. In the other hand there was a significant decrease in total serum IgM. FNT showed variable histopathological alterations in liver, kidney and gills depending on the dose level of FNT exposure. Considering these results, it can be concluded that oxidative stress evoked by FNT could be responded of its genotoxicity which was proven by determined clastogenic effect resulting from over production of reactive oxygen species (ROS) or depletion of endogenous antioxidants.

**Key words:** Fenitrothion · *Oreochromis niloticus* L. Oxidative stress · SCGE assay · LC₅₀

**INTRODUCTION**

Organophosphothionate(OP) insecticide fenitrothion; FNT (O, O-dimethyl, O-4-nitro-methyl phosphorothioateis usedas contact insecticide, effective against a wide range of pests on rice, cereals, fruits, vegetables, stored grains, cotton and forests and also in public health programs as a vector control agent for malaria, flies, mosquitoes and cockroaches. FNT is produced at the rate of 15,000 to 20,000 tons per year worldwide [1]. In general, FNT is considered moderately toxic to fish and known as potential toxic pollutant contaminating aquatic ecosystems [2]. Aquatic organisms (fish and invertebrates) are potentially at risk, especially in the event of overspray to static water bodies. FNT is usually sprayed aerially were the pesticide may drift near rivers and ponds [3]. Freshwater systems are often exposed to pollution with OP insecticides which is mostly utilized in agricultural practices, this also creating potential danger for non-target organisms exposed to either lethal or sub lethal concentrations of these contaminants [4].

The primary effects of OPs on organisms are through the inhibition of acetylcholinesterase (AChE), the enzyme responsible for terminating the transmission of the nerve
impulse. However, there is evidence that oxidative stress may be implied in OP toxicity more than AChE inhibition, since [5] found that different classes of pesticides may induce in vitro and in vivo generation of Reactive Oxygen Species (ROS), which react with biological macromolecules and produce enzyme inactivation, lipid peroxidation and DNA damage [6]. The production of reactive oxygen species has long been regarded as a possible mechanism of pesticide-induced toxicity as evidenced by triggered oxidative stress in a number of studies [7]. The toxic effects of FNT probably occur through the generation of Reactive Oxygen Species (ROS) causing damage to various membranous components of the cell [8].

Since presence of genotoxins in the aquatic environment is a well-recognized fact, attempt to develop sensitive biomarkers to evaluate genotoxic effects in aquatic organisms has gained importance. The effects of pesticides on DNA integrity have been reported in studies in which comet assay has been successfully used in different fish species [9].

Nile tilapia (Oreochromis niloticus L.) is a widely used aquaculture species worldwide [10] and has been termed the aquatic chicken” for its extraordinary production capabilities [11]. Nile tilapia has been the most popular species in Egypt. Although there are some studies on biochemical effects of FNT, there is little data obtained about FNT toxicity in fish and a lack of data for the genotoxic and harmful effects of sublethal concentrations of FNT on Nile tilapia. For these reasons, O. niloticus L. fingerlings were chosen as a model in the present study to evaluate the 96 h median lethal concentration (LC$_{90}$) of FNT. Serial dilutions of FNT were prepared. All groups of tested fish were exposed individually for 96 h to different serial dilutions of FNT as shown in Table 1. Mortality was checked at 24, 48, 72 and 96 h after the start of the experiment. Dead fishes were removed immediately. Behavioral changes were followed closely.

The second experiment was done to determine the sublethal effects of FNT acute exposure. Acute 2 sublethal concentrations of 2.35 mg/L and 1.566 mg/L were used that corresponding to ½ and 1/3 of LC$_{90}$ value (Calculated in the present study), respectively. After acclimatization, Nile tilapia (Oreochromis niloticus L.) fingerlings (n= 90) with 10.14-10.40 g mean body weights were randomly divided into three groups each with 30 individuals. Each group with two replicates containing fish at density of 15 fish per aquarium. Group 1 was reared in pesticide free tap water and treated as control. Fish belonging to group 2 and 3 were exposed to the mentioned sub lethal concentrations of FNT respectively for 96h. Aquaria water was completely changed twice weekly to maintain water quality with the appropriate pesticide amount.

Blood samples of fish from all groups were taken from caudal vein and processed immediately according to [12]. Sera were separated and immediately frozen at-80 for further estimations of biochemical parameters. For histopathological examination, after the end of the experimental period, fish were killed immediately and the tissues (liver, kidneys and gills) were collected and fixed in 10% buffered neutral formalin solution and then passed to 70% ethanol, dehydrated through graded ethanol series (70-100%), cleared in xylene and embedded in paraffin. Five micron thick paraffin sections were cut and stained with haematoxyline-eosin (H&E) and examined under a light microscope [13].

### Abbreviations:
- FNT: fenitrothion
- SOD: superoxide dismutase
- MDA: malondialdehyde
- SCGE: single cell gel electrophoresis
- GSH: reduced glutathione
- TM: tail moment
- IgM: immunoglobulin M.

### MATERIALS AND METHODS

**Experimental Materials and Procedures:** Nile tilapia (Oreochromis niloticus L.) fingerlings (n= 240) were obtained from the ALAbassa fish farm, Sharkia province with average weights were (9 ± 0.5 g). Fishes were randomly divided into experimental aquaria (80X 30X40 cm) and acclimated for laboratory conditions for 10 days (10 °C/aquarium). During acclimatization, fish were held in permanently aerated tap water. Water renewal was made twice weekly. FNT used in this study was of analytical standard grade (CAS number:122-14-5) and purchased from Sigma-Aldrich Chemical Corporation (Egypt). All the other chemicals were of analytical grade and obtained from Sigma-Aldrich (Egypt).

For the first experiment, Nile tilapia (Oreochromis niloticus L.) fingerlings (n= 150) were used to determine the 96h median lethal concentration LC$_{90}$ of FNT. Serial dilutions of FNT were prepared. All groups of tested fish were exposed individually for 96 h to different serial dilutions of FNT as shown in Table 1. Mortality was checked at 24, 48, 72 and 96 h after the start of the experiment. Dead fishes were removed immediately. Behavioral changes were followed closely.

The second experiment was done to determine the sublethal effects of FNT acute exposure. Acute 2 sublethal concentrations of 2.35 mg/L and 1.566 mg/L were used that corresponding to ½ and 1/3 of LC$_{90}$ value (Calculated in the present study), respectively. After acclimatization, Nile tilapia (Oreochromis niloticus L.) fingerlings (n= 90) with 10.14-10.40 g mean body weights were randomly divided into three groups each with 30 individuals. Each group with two replicates containing fish at density of 15 fish per aquarium. Group 1 was reared in pesticide free tap water and treated as control. Fish belonging to group 2 and 3 were exposed to the mentioned acute sub lethal concentrations of FNT respectively for 96h. Aquaria water was completely changed twice weekly to maintain water quality with the appropriate pesticide amount.

Blood samples of fish from all groups were taken from caudal vein and processed immediately according to [12]. Sera were separated and immediately frozen at-80 for further estimations of biochemical parameters. For histopathological examination, after the end of the experimental period, fish were killed immediately and the tissues (liver, kidneys and gills) were collected and fixed in 10% buffered neutral formalin solution and then passed to 70% ethanol, dehydrated through graded ethanol series (70-100%), cleared in xylene and embedded in paraffin. Five micron thick paraffin sections were cut and stained with haematoxyline-eosin (H&E) and examined under a light microscope [13].
Analysis

Comet Assay (SCGE): After the end of the experimental period fish were killed immediately and the tissues of gills were immediately taken, washed with phosphate buffer saline then kept at-80 for determination of DNA damage by alkaline comet assay according to [14].

Oxidative Stress Biomarkers: Serum samples were used for detection of Superoxide dismutase (SOD) activity according to the method described by [15], Malondialdehyde (MDA) concentration according to the method adapted by [16], reduced glutathione (GSH) content according to the method described by [17].

Serum Biochemical Parameters: Serum alanine aminotransferase (ALT) was determined colorimetrically according to method of [18] and modified by [19], serum creatinine according to method adapted by [20], serum urea level according to the method described by [21] serum cortisol according to the method described by [22]. Finally, IgM levels were determined according to the method described by [23]. Protein levels estimation were determined by the method of [24] using bovine serum albumin as standard.

Statistical Analysis: Statistical analysis was based on comparing the values between the untreated control group with the low and high acute FNT exposed groups. The results are expressed as means ± SD. The statistical significance of the data has been determined using one way analysis of variance (ANOVA-LSD) using the statistical package for social science [25]. The level of significance was taken as (P< 0.05).

RESULTS AND DISCUSSION

Experiment 1: Determination of 96 h LC₅₀ value of FNT: In the current experiment, preliminary acute toxicity test (exposing Oreochromis niloticus fingerlings to FNT), allowed the determination of 96 h LC₅₀ value of FNT was 4.7 mg/L. Control mortality was zero. Results are shown in table (1). Concerning the estimation of FNT 96 h LC₅₀ value in Nile tilapia fingerlings, the results of existing study showed that FNT 96 h LC₅₀ in Nile tilapia fingerlings is about 4.7mg/L. This estimate indicates that FNT is highly toxic to Nile tilapia. These findings disagree with the results previously obtained [2] where the 96 h LC₅₀ was 0.84 mg/L in M. galloprovincialis and Oreochromis niloticus fingerlings. This difference may be attributed to species differences, different inborn capacity of each species to detoxify pollutants and different tolerance to FNT, or may be ascribed to individual, seasonal and environmental variations or may be qualified to differences in the development stage and size of fish used or different basal activities of key enzymes of FNT detoxification.

Experiment 2: Determination of the sublethal toxic effects of FNT acute exposure on Nile Tilapia fingerlings.

Behavioral Changes: Observations of behavioral response after FNT acute exposure in Nile tilapia Oreochromis niloticus fingerlings conducted at first two hours and every 12 h during the acute toxicity tests. Regarding the control group, fish showed normal behavior. Fish of FNT acute exposure exhibited shifts in behavior in the current study which differs depending on dose of FNT exposure. Deviations in behavior were more intense in high exposure level compared with low one. The changes includes rapid gill movements, loss of equilibrium, loss of escape reflex, much decreased general activity and lying motionless on the bottom on their backs. In relation to behavioral changes, our results are in agreement with that obtained [2]. The nervous system is important to the most of the physiological processes and the mechanisms essential for behavior [26]. Behavioral alterations are associated with nervous system impairments. Many pollutants, particularly pesticides, have high potential to directly alter behavior, since they are neurotoxins. FNT an OP insecticide was known to cause neurotoxic effects on target and non-target organisms through inhibition of AChE activity [27]. Recent results suggest that cholinesterase inhibitors can induce sub lethal effects on a variety of parameters with implications for organisms’ fitness [28]. Furthermore, Alterations in behavior obtained in our study may be one of the major mechanisms by which animals adapt to changes in their environment, including exposure to contaminants as mentioned by [29]. Also, changes in behavior considered as a useful biomarkers to assess chemical exposure and/or effect [30].

Oxidative Stress Biomarkers: Relating to the effect of acute and chronic low and high sublethal intoxication of Nile tilapia fingerlings to FNT insecticide on oxidative stress parameters, the data demonstrated in Table (2) declared a significant depletion of serum reduced glutathione content (GSH), significant elevation in the serum SOD activity and in lipid peroxidation biomarker (MDA) after FNT insecticide exposure versus control groups at p<0.05. Associating with oxidative stress
Table 1: Actual evaluation of 96 h median lethal concentration (LC₅₀) of FNT insecticide in Nile tilapia Oreochromis niloticus L. fingerlings

<table>
<thead>
<tr>
<th>Group</th>
<th>Conc. Mg/L</th>
<th>No. of fish</th>
<th>No. of fish after 96 hr.</th>
<th>a</th>
<th>b</th>
<th>a X b</th>
<th>Σ (a X b)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Zero</td>
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<tr>
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<td>1.5</td>
<td>10</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
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<tr>
<td>5</td>
<td>2</td>
<td>10</td>
<td>2</td>
<td>0.5</td>
<td>1.5</td>
<td>2</td>
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<td>1</td>
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<td>4</td>
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<td>0.5</td>
<td>4</td>
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<td>0.5</td>
<td>4.5</td>
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<td>6.5</td>
<td>10</td>
<td>7</td>
<td>0.5</td>
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<td>7</td>
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<td>8</td>
<td>0.5</td>
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<tr>
<td>16</td>
<td>7.5</td>
<td>10</td>
<td>8</td>
<td>0.5</td>
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<td>4</td>
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<td>17</td>
<td>8</td>
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<td>9</td>
<td>0.5</td>
<td>8.5</td>
<td>4.25</td>
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<tr>
<td>18</td>
<td>8.5</td>
<td>10</td>
<td>10</td>
<td>0.5</td>
<td>9.5</td>
<td>4.75</td>
<td>38</td>
</tr>
</tbody>
</table>

a= Constant factor between two successive doses b= the mean of dead fish in the groups. n= Number of fish in each group. Σ= the sum of (a X b).

As regards to MDA level a marker of lipid peroxidation, (LPO), the present study indicated a significant increase in MDA concentration in FNT exposed fish compared with control ones. These findings are concordant with that obtained by [34]. Lipids are the main cellular components susceptible to damage by free radicals (peroxidation of unsaturated fatty acids in cell membrane) and nucleic acids. LPO is a well-recognized mechanism of cell damage and considered as one of the molecular mechanisms involved in pesticide toxicity and its predictive importance as a biomarker for oxidative stress is indicated in different investigations [35]. OP pesticides can lead LPO either by direct interaction with cellular plasma membrane [36] or reactive oxygen accumulation caused by excites toxicity triggered by their anti-cholinesterase activity [37].

Concerning the serum SOD activity, the current study revealed significant increase in serum SOD enzyme activity in FNT exposed Nile tilapia compared with control one. SOD is one of the most important defense biomarkers, the findings in our experiment are coincident with that obtained by [31], which supporting the occurrence of oxidative stress induced by FNT intoxication. Concerning GSH content, the observed depletion in the glutathione GSH is considered as an early consequence of FNT induced oxidative stress as GSH molecules scavenges free radicals resulting from oxidative metabolism or that are escaping from detoxification by antioxidant enzymes [32]. Consequently depletion in GSH content in this study is due to oxidation of GSH to glutathione disulfide GSSG by free radicals produced by FNT insecticide. Also, depletion of GSH may be attributed to detoxification process depending on glutathione-S-transferase (GST) enzymes which is one of enzyme system consuming GSH molecules as a substrate in the detoxification of OP insecticides to non-toxic products or by rapidly binding and very slowly turning over the insecticide [33]. Augments in GSH levels demonstrate a protective response in fish towards exposure to an oxidative-stress-inducing xenobiotic, such as FNT.

Table 2: Changes in serum reduced glutathione GSH content (ng/ml), superoxide dismutase SOD activity (unit/mg protein) and malondialdehyde MDA (nmol/ml) concentration of fish after acute exposure to high and low concentrations of FNT (Mean± S.E.)

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Acute high FNT exposed group</th>
<th>Acute low FNT exposed group</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (ng/ml)</td>
<td>9.91±0.3 a</td>
<td>2.92±0.09 c</td>
<td>5.33±0.21 b</td>
</tr>
<tr>
<td>SOD (unit/l)</td>
<td>48.31±1.35 c</td>
<td>90.79±2.9 c</td>
<td>81.41±1.37 b</td>
</tr>
<tr>
<td>MDA (nmol/l)</td>
<td>33.21±0.44 d</td>
<td>64.51±0.93 c</td>
<td>41.99±2.60 b</td>
</tr>
</tbody>
</table>

Means in the same row having different superscript letters were significantly different (P< 0.05)
Table 3: Oxidative DNA damage (comet assay) observed in gills of Nile tilapia fingerlings after acute exposure to FNT two sub lethal concentrations (Mean±S.E.)

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Acute high FNT exposed group</th>
<th>Acute low FNT exposed group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail length (px)</td>
<td>15.83±1.6^c</td>
<td>37.3±1.4^a</td>
<td>41.8±0.8^b</td>
</tr>
<tr>
<td>Tail DNA%</td>
<td>0.313±0.04^a</td>
<td>1.088±0.11^b</td>
<td>2.108±0.33^a</td>
</tr>
<tr>
<td>Tail moment</td>
<td>0.122±0.02^a</td>
<td>0.743±0.15^b</td>
<td>0.346±0.04^b</td>
</tr>
</tbody>
</table>

Means in the same row having different superscript letters were significantly different (P<0.05)

Fig. 1: Nucleus of gill cells of Nile tilapia fingerlings after acute exposure to low and high FNT sub lethal concentrations
A) control group almost normal condensed type nucleus B) acute high FNT exposed group C) acute low FNT exposed group.

Evaluation of DNA Damage via Comet Assay: In the existent study, intoxication of FNT insecticide in Nile tilapia fingerlings resulted in DNA damage as indicated by the significant increase in tail length (px), tail DNA% and TM in groups of FNT intoxicated fish compared with the control ones in these experimental circumstances as presented in Table (3) and Figure (1). Group exposed to low sub lethal acute FNT intoxication showed non-significant increase in tail moment than the control group at p<0.05. Regarding to the effect on DNA, The single cell gel electrophoresis (SCGE), known as comet assay, is recognized as one of the most sensitive and reliable methodologies available for DNA strand break detection with the advantages of being fast, simple and applicable to any eukaryotic cell type in vivo as well as in vitro [41]. SCGE or comet assay is used as a strong tool evaluating relationship between DNA damage and exposure to genotoxins in genetic toxicology. The percent DNA in the tail reflecting amount of DNA migrated out of nucleus is strongly recommended as the parameter of choice and directly linked to DNA break frequency. The other endpoints such as TM derived from % Tail DNA and preferred in sensitive quantification of DNA damage [42]. Observed high DNA damage in Nile tilapia fingerlings after FNT insecticide exposure in the present research confirms that comet assay using fish is effective in determination of genotoxic effects of chemicals [43]. Additionally, revealed positive correlation between acute sub lethal FNT exposure and TM resulted in TM to be considered as an endpoint providing more accurate reflection of DNA damage in this study. FNT as an OP, requires metabolic activation to exert its toxic effects, however induction of oxidative stress and increase in DNA damage were reported in in vitro studies conducted with OP pesticides [44]. DNA damage in this study is explained by occurrence of reactive oxygen species during metabolism of the pesticide and it is suggested that reaction between reactive species and DNA resulted in lesions determined by comet assay. Alkylation property of OP is proposed as another mechanism by which alkylation of DNA bases is involved in DNA damage [45]. The electron-rich atoms in DNA are readily attacked by electrophiles and transfer of methyl, ethyl or alkyl group causes phosphorylation or alkylation resulting in mutagenic or clastogenic effects [46]. Finally, DNA damaging effect of FNT detected in gills of Nile tilapia mechanisms against toxic effects of oxygen metabolism. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide therefore; maintain low steady-state concentrations of the ROS and alleviate their toxic effects [38]. Taking this into account, pro-oxidant conditions elicited by pesticides could trigger increases in the activity of this antioxidant enzyme as an adaptive response [39]. On the other hand, the activity of the antioxidant enzymes could be increased or inhibited by xenobiotic exposure depending on the intensity and the duration of the stress applied, as well as the susceptibility of the exposed species [40]. Induction of SOD activity observed in our study may be attributed to the high production of superoxide anion radical after FNT insecticide exposure.
fingerlings may be attributed to both oxidative stress producing potential, as confirmed with altering antioxidant enzyme activities and increased lipid peroxidation in this study and alkylating property of this pesticide.

**Serum Biochemical Parameters:** The recorded results of the present study as shown in Table 4 revealed liver damage which is indicated by a significant increase in the serum levels of ALT in FNT exposed groups than the control ones. Furthermore, there was significant increase in serum levels of cortisol, creatinine, urea, significant decrease in IgM of fish groups after acute exposure to FNT insecticide compared with the control ones at p<0.05. Concerning the toxic effect of FNT insecticide on liver enzymes, our study reported a significant increase in serum ALT enzyme. This result coincides with that obtained by [47]. That showed a significant increase in liver enzymes after exposure to OP insecticides FNT and chlorpyrifos. Previous in vivo studies showed that this compound had a significant increase in serum ALT and AST activities and serum level of total protein, creatinine and urea compared with that of the control level of rat. ALT and AST are enzymes used as indicators of FNT hepatic damage to rat liver tissue [48]. Or may be due to alterations in the permeability of cell membrane and increased synthesis or decreased catabolism of aminotransferases [49]. Generally, elevated ALT enzyme level may indicate degenerative changes and hypofunction of liver as the toxicants effects on the hepatocytes are in the form of tissue damage in which cellular enzymes are released from the cells into the blood serum which gives an indication of the hepatotoxic effect of toxicants [50]. The above findings were confirmed by the obtained histopathological changes in liver and kidney under the intoxication effect of FNT in the present study.

Concerning to cortisol level, the obtained data in the existent investigate showed significant increase in the serum cortisol level. Cortisol assessment generally has been used as a sign of stress response in fish [51]. The increase in blood glucose concentrations is known as a general secondary response to stress of fish to acute toxic effects and is considered as a reliable indicator of environmental stress [52]. The stress hormone cortisol has been shown to increase glucose production in fish, by both gluconeogenesis and glycogenolysis and likely play an important role in the stress-associated increase in plasma glucose concentration [53]. In regards to the increase in urea and creatinine may be due the decrease of glomerular filtration rate of kidney and tubular dysfunction [54] which is confirmed by the obtained significant histopathological injury in kidneys of Nile tilapia after FNT insecticide exposure.

In respects to IgM, our search demonstrated that there is a significant decrease in total circulating IgM. This result is disagreed with that mentioned by [55] who recorded that after acute exposure to the pesticide, the hepatic biochemical parameters and the total circulating IgM concentrations were not affected. This may be attributed to the toxic effect of FNT insecticide.

**Histopathological Findings:** Liver of control group showed normal liver architecture with the central vein and radiating cords of normal hepatocytes with central rounded nuclei. Normal blood sinusoids appeared between the liver cords (Fig 2.1). Concerning to FNT exposed fish; hepatocytes showed diffuse fatty changes and coagulative necrosis which is more severe in high level of FNT exposure (Fig 2.2). Mononuclear aggregations in the portal areas and interstitial tissue were seen (Fig 2.3). Extensive hemorrhage and congestion with sinusoidal enlargement were also visualized. Concerning to kidney, in the control group revealed normal cortex showing normal renal corpuscles with Bowman’s capsules and renal glomeruli are made of tuft of blood capillaries. Sections of the proximal and distal convoluted tubules showed normal cuboidal epithelial lining (Fig. 3.1). Extensive hemorrhage and congestion with sinusoidal enlargement were also visualized. Concerning to gills of Nile tilapia

### Table 4: Changes in serum ALT, cortisol, creatinine, urea and IgM of fish after acute exposure to high and low concentrations of FNT (Mean± S.E.)

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Acute high FNT exposed group</th>
<th>Acute low FNT exposed group</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/dl)</td>
<td>13.67±0.33b</td>
<td>17.67±0.33b</td>
<td>16.00±0.57b</td>
</tr>
<tr>
<td>Cortisol (m/dl)</td>
<td>6.14±0.05c</td>
<td>9.47±0.43b</td>
<td>8.49±0.08b</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.15±0.005c</td>
<td>0.73±0.01c</td>
<td>0.63±0.01c</td>
</tr>
<tr>
<td>Urea (IU/dl)</td>
<td>7.67±0.33c</td>
<td>16.00±0.57b</td>
<td>13.67±0.33b</td>
</tr>
<tr>
<td>IgM value(µg/ml)</td>
<td>23.38±0.33c</td>
<td>13.14±0.70b</td>
<td>15.33±0.67b</td>
</tr>
</tbody>
</table>

Means in the same row having different superscript letters were significantly different (P< 0.05).
fingerlings, the gills of control groups showed normal filaments and respiratory epithelium (Fig 4.1). The gills of acute exposure to FNT revealed focal epithelial and mucous cells proliferations, fusion and few leukocytic infiltrations (Fig 4.2). Focal sloughing of the secondary lamellae with aggregations of lymphocytes and congestion lamellar capillaries were observed (Fig 4.3). Diffuse proliferation and fusion of the respiratory epithelium with hemorrhage and leukocytic infiltration (Fig 4.4). The present study revealed that FNT insecticide showed varying pathological injuries depending on the dose of exposure. These findings support the results of the study which stated that FNT was found to induce ultra-structural changes in liver cells where nuclear membrane was completely distorted. Nuclear intactness was totally lost and smooth endoplasmic reticulum and Golgi apparatus was abnormally enlarged after 24 h of intoxication [56]. This can be explained by the fact that FNT administration is associated with alteration of the ratio of various components of phospholipids and neutral lipids in various organs of rats up to 48 h. These changes in relative composition of various lipids by FNT administration may lead to malfunctioning and alteration of biological properties [57]. Liver being the main organ of various key metabolic pathways, toxic effects of chemicals usually appear primarily in the liver. This, in turn provides important data on the chemical’s toxicity and mode of action. Many organic compounds induce toxicopathic lesions in the liver of fish species. The histopathological changes in kidneys and gills may be attributed to the generation of oxidative stress and consequent lipid peroxidation occurred by pesticides which is reported in many species. Due to high concentration of polyunsaturated fatty acids in cells, lipid peroxidation is a major outcome of the free radical mediated injury. Two broad outcomes of lipid peroxidation are structural damage of cellular membranes and generation of oxidized products. These reactive products are thought to be the
major effector of tissue damage from lipid peroxidation as recorded by [58]. The same mechanisms operate in the kidney as FNT can cause nephrotoxicity due to increased lipid peroxidation and decreased antioxidant potential by increasing oxidative stress.

CONCLUSION

These results indicate that oxidative stress biomarkers are highly sensitive to FNT exposure and these biological responses in Nile tilapia Oreochromis niloticus L. fingerlings could be valuable biomarkers to monitor OP and other pollutants contamination in aquatic environments.

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REFERENCES


