FTIR study of the binary effect of titanium dioxide nanoparticles (nTiO2) and copper (Cu$^{2+}$) on the biochemical constituents of liver tissues of catfish (*Clarias gariepinus*)

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**ABSTRACT**

The increasing demand for nanomaterials and essential metals leads to their discharge into the aquatic ecosystems through water run-off and this is of great concern for the aquatic biodiversity. In the present study, titanium dioxide nanoparticles (nTiO2) and copper (Cu$^{2+}$) effects in binary mixture on the biochemical constituents of the liver of *Clarias gariepinus* by using Fourier-transform infrared (FT-IR) techniques was examined. The FT-IR revealed significant differences in absorbance intensities between the control and exposed liver tissues demonstrating changes on the critical biochemical constituents such as proteins, lipids and carbohydrates. This result further reveals the binary mixture responsible for the absence of CH$_3$ bending lipids in liver tissues due to their toxic effect. The observed synergistic decreasing ratio of integrated area (1545/3296) for binary mixture exposed liver tissues suggests that lipid degradation predominates over amide formation. In addition, binary mixture causes an alteration in protein secondary structures by decreasing the β turns of liver tissues. Histology in liver showed marked damages. The frequent alteration in the biochemical constituents in the liver tissues of *C. gariepinus* could be an indication of alteration of existing biochemical components or the expression of new components.

**1. Introduction**

The global production and consumption of nanomaterials per annum is on the rise because of the population high demand of manufactured products [42]. Nanomaterials made up of nanoparticles, nanotubes, nanoporous material, quantum dots nanostructures, nanofibers and nanowires are used in a myriad of applications in medical/pharmaceutical, chemicals and advanced materials, information communication technology, energy, automotive, aerospace, textiles and agriculture [42]. The large production of nanomaterials inevitably leads to their released into the environment with harmful consequences on several aquatic organisms [31,41]. However, the production of titanium dioxide nanoparticles nanomaterial is estimated at 550–5500 million tons per year, making it the most prominent produced engineer nanomaterial worldwide [38]. Human activities, climatic and environmental phenomena are responsible for their discharge into aquatic ecosystems. Available data is scarce on the discharged of nTiO$_2$ into surface waters [18]. However, the predicted concentration of nTiO$_2$ in aquatic ecosystem and rivers range from ng to μg. L$^{-1}$ [13]. The presence of nTiO$_2$ in freshwater ecosystem has become a great concern for the aquatic biodiversity including fish spp. In natural environment nTiO$_2$ has been reported to enter the blood circulation leading to the disruption of reproduction, liver, brain and many other organs and tissues damage [48].

The sublethal concentrations of nTiO$_2$ have been reported to have harmful effect on the physiology and reproduction of zebrafish [15]. Toxic effects of nTiO$_2$ on fish include damage of gills epithelium, the brain and oxidative stress in *Onchorhyncus mykiss* exposed to 1 mg.L$^{-1}$ for 14 days [11,39,48]. Lipid peroxidation, catalase reduction and mortality were observed in Carp (*Cyprinus Carpio*) and Zebrafish exposed to nTiO$_2$ [7,16].

However, in aquatic environment nTiO$_2$ inevitably interacts with other metal which can bioaccumulate in fish; as a result the mixture alter the tissues, the bioavailability of the metal, the biochemical composition of tissues and the toxicity of fish [40,55]. Copper is an essential metal naturally found in rocks scattered through the environment by processes known as geological, meteorological or biological [1,3]. However, literatures elucidating the critical effects of this essential metal (Cu) in the presence of nTiO$_2$ are limited. Few studies demonstrated that nTiO$_2$ in freshwater ecosystems adsorb co-occurring stressors such as Cr (III), Mn(II), Ni (II), Cd (II) and Mo (VI) [19]. Nonetheless there is paucity of information on the interaction...
between nTiO₂ and essential metal such as Copper in fish.

About 15 million tons of Cu is used worldwide and can bio-concentrate into the environmental compartment of the ecosystems and remain potentially toxic to the biodiversity [24]. Aquatic ecosystems are then considered susceptible to Cu pollution because they are dumping ground for all sort of waste, mining and industrial debris. Elevated concentrations (4–4600 ppb and 325 ppb) around mining and urban storm water runoff respectively are reported lethal to aquatic biodiversity [2,43]. However, copper could be lethal to freshwater fish species exposed to concentrations less than 10 Part per billion (ppb) in natural freshwater [21]. Copper causes irreversible damage on the growth, smell orientation, reproduction, immune response, gills and dietary receptor, disrupt osmoregulation structure, pathology of kidney and liver of fish [8,10,47].

In this regard nTiO₂ with known specific physiological properties such as insolubility, large surface area to volume ratio is likely to interact with copper in freshwater ecosystem and alter the biological function of freshwater fish. Interaction between Cu and nTiO₂ is therefore of concern for the risk of biodiversity including freshwater fish C. gariepinus. To address and fill this knowledge gap the effects of addition of Cu to nTiO₂ is relevant in order to have an insight on the effect of their interaction on the biochemical composition on freshwater fish tissues in natural ecosystem.

Analysis of effluents water discharged in natural ecosystems such as stream revealed significant levels of nTiO₂ substances according to [50], the concentrations varied between 27–43 μg/L. [2] studies revealed the concentration of Cu in the lower Sinos river in Brazil, the measured concentrations in water and sediments varied between 5.5–12.1 and 8.6–15.1 μg/g wet weight respectively.

The levels of Cu and nTiO₂ continuously increase in aquatic environment because of their increasing demands by human. It is known that, individually, there are able to disturb the physiology and histology of aquatic organisms. Studies have assessed the impact of these pollutants individually and have reported liver pathology [14–16,25]. Therefore it is very important to describe the binary effects of Cu and nTiO₂ at molecular level and the histopathology of fish. Despite several biochemical studies, the studies reporting the pathological effects of mixed Cu and nTiO₂ on tissues and studies at molecular level are very limited. A few studies have revealed the effects of individual nTiO₂ on the biochemical constituents of gill tissues of zebra fish [14,32].

Clarias gariepinus is one of the most commonly cultures and consumed freshwater fish in Africa. The presence of metals or xenobiotics compounds in fish liver could stimulate the disruption of protein enzymes, fats and carbohydrates, it is also known as the site of storage of nutrient that sustains fish when food resources are scarce. Studies have revealed that environmental metal pollutants have been found to cause alteration of the liver in fish with pathology ranging from necrosis to apoptosis of hepatocytes [16].

For this reason, the current study was firstly conducted in order to particularly determine the histopathological effects of binary mixture of Cu and nTiO₂ on the liver tissues of C. gariepinus. As biochemical alterations are likely to proceed, or at least be concomitant with the histological changes [32].

Hence, the histopathological effects of Cu and nTiO₂ on liver tissues, the changes in the biochemical constituents and the protein structural changes were investigated at molecular level using FTIR spectroscopy.

Fourier-transform infrared (FTIR) spectroscopy is known biophysics tool for biochemical analysis to characterize the structures of proteins, lipids, carbohydrate and nucleic acid and provide information from all tissues components. It is an important technique to study the changes at molecular level in biological samples.

FTIR is vibrational spectroscopic techniques commonly used because of its straightforwardness [37]. Moreover, this method is rapid sensitive and easy to perform and provide a precise measurement that does not use external calibration [9,27]. In this method, the samples can be studied in any state and short time to obtain data. An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. The size of the peak in the spectrum is also a direct indication of the amount of macromolecules present [30]. The technique requires both minimal sample preparation and minimum amount of analyte as compared to traditional chromatographic techniques like HPLC, HPTLC and GC.

Among the freshwater fish, in Nigeria, Catfish C. gariepinus has been selected for the present study because of its nutritional values composition and it can withstand wide range environmental conditions. C. gariepinus is a favourite food fish in Africa, there may be concern for metal and nanoparticles uptake by fish and human. Human in Africa predominantly consume fish with their offal’s such as liver, with the assumption that the entire fish is rich in nutrient.

2. Materials and methods

2.1. Test species

The adult freshwater fish C. gariepinus (length: 42.33 ± 585 cm, weight: 464 ± 66.9 g) were procured from Kado fish Farming company, Gwarinpa, District, Abuja, Nigeria. Fish were transported in aerated container with pond water to the Laboratory. Fish were transferred in glass aquaria for acclimatization period of two weeks and at 25 ± 2 °C, 12 h/12 h light/dark cycle. During this experiment the fish were fed with Coppens® pelleted food for Catfish.

2.1.1. Test chemicals

Titanium dioxide nanoparticles (purity 99%, anatase-rutile, size: 21 nm) and copper sulphate (CuSO₄) salt were purchased from Sigma-Aldrich (St Lois, Mo, USA).

2.2. Particle characterization

Prior to the experiment, X-ray diffraction analysis was carried out using X-ray Diffractometer (Xpertpro, Panalytical, Phillips) (Fig. 1) at the Panafrican University of Technology, Abuja, Nigeria. Zeta-potential was determined using the Zetaplus (Brookhaven 22001) after dispersion of the nanoparticles in physiological saline and ultrasonicated (Ultrasonic Cleaner, SB-4200DTD) for 15 min at 25 °C, 40 Hz frequency.

2.3. Titanium and Copper sulphate stock solution and preparation

A stock solution of 3 mg L⁻¹ of nTiO₂ was prepared by dispersion nanoparticles in physiological saline as reported by [20,40], and sonicated for 15 min in a sonicator. The stock solution of CuSO₄ was also prepared by dispersing 2.5 mg of CuSO₄ in 1 L of physiological saline. The experimental concentration used in this study was obtained after a serial dilution.

2.4. Experimental study

The sub-lethal concentrations used in this design study were those reported by [52] and [54] on Catfish C. gariepinus. Both concentrations were used in this study because there were not susceptible to cause the death of fish after a long period of time (2 weeks), according to the [55], this study period was referred as chronic study. The fish acclimated in this study were divided into four (4) groups (G I, G II, GIII and G IV) and three replicates each containing five (5) fish and rear in dechlorinated water. In this study G I was used as the control, whereas G II, G III and G IV were subjected to caudal peduncle intravenous injection which consist of injecting the fish with 5 ml seringe in a horizontal direction on the lateral line of the tail [53] of Cu (250 μg L⁻¹), nTiO₂ (3 μg L⁻¹) and binary mixture of Cu (250 μg L⁻¹) + nTiO₂ (3 μg L⁻¹) respectively. After a period of 2 weeks, the fish were sacrificed, dissected and the liver was extracted and stored at
-80 °C prior to FTIR spectroscopy.

2.5. Histopathology

Selected liver tissues were prepared for histopathology. Tissues were fixed in buffered 10% neutral formalin, dehydrated, embedded in paraffin and sectioned on a microtome at thickness of 4 μm and stained with hematoxylin and eosin [26,44].

2.6. Sample preparation

The liver tissues extracted were freeze-dried (Heto-Maxi) for 10 h to remove water content in the samples. The samples were ground in a mortar using a pestle and the powder obtained (5 mg) was mixed with potassium bromide (KBr) (100 mg). The mixture was then compacted at a pressure of 9000Psi for 5 min. The disc obtained after pressure was transparent and about 12 mm and 1 mm thickness used for FTIR readings.

2.7. Spectroscopic measurement (FTIR)

In this study the measurement of spectra was performed in the region 4000-400 cm\(^{-1}\) on a Nicolet iS5 in built with a detector DTGs (Thermo Scientific) using the KBr pellets. A spectrum was taken as the average of 160 scans to increase the signal of the noise ratio and spectral resolution of 4 cm\(^{-1}\) and accuracy of frequency of band to 0.01 cm\(^{-1}\). The absorption intensity of the peaks in this study was determined using the baseline methods. The variations in the frequencies and band areas were determined accurately from the original baseline corrected spectrum to the corresponding control and treated samples. In this study it was also logical to directly relate the intensities of the absorption bands to the concentration of corresponding functional groups [45]. All spectral manipulation was performed using the Origin 8.0 software for window.

2.8. Accumulation of TiO\(_2\) NPs and Cu\(^{2+}\)

Accumulation of copper was determined using the scientific Nov 300 Atomic Absorption Spectrophotometer. The calibration plot method was used for analysis. The air acetylene was the flame used and hollow cathode lamp of the corresponding element was the resonance. Line source, the wave length for the determination of copper was 324.7 nm and 353 for Titanium [56].

2.9. Statistical analysis

The difference in the means of the exposed fish and control values were compared by means of ANOVA; p values less than 0.05 were considered significant. The results in this study were expressed as mean ± standard deviation.

3. Results and discussion

Titanium dioxide nanoparticles have been previously reported to cause histopathological distortion in freshwater fish exposed to xenobiotic compounds including nanomaterials [51].

Histological observations from [16] demonstrated that nTiO\(_2\) caused pathological changes in juveniles carp (Cyprinus carpio). Elsewhere studies on histopathology of common carp also showed that copper could cause morphological alteration of the liver [45].

The liver tissues are known to play an important role on active metabolism of carbohydrates, proteins, lipids, amino acid and so forth but also play a crucial role in detoxification in fish. Xenobiotics compounds such as non essential metals of sub-chronic concentrations could be found bioaccumulated and adulterating the architecture of the liver of fish after some period of exposure [28].

Hao et al. [16] reported that the livers of some fish (Cyprinus carpio) exposed to nTiO\(_2\) showed cellular pathologies such as cytoplasm vacuolation and apoptosome including necrotic cells. However, sub-chronic exposure of copper to C. carpio caused marked dystrophic lesions of hepatocytes, with morphological signs of cell injury included the hypopic to vacuolar degeneration of hepatocytes, the dilation of...
capillaries, and cholestasis.

In our study (Fig. 2), exposure to individual concentration of copper caused alteration of livers’ morphology and revealed focal bile duct proliferation with hepatic vacuolation. Multifocal hepatic necrosis with hepatic vacuolation around the central vein of the liver of *C. gariepinus*. Furthermore, titanium nanoparticles and combination (Cu + nTiO$_2$) exposed to fish revealed morphological changes represented by multifocal hepatic necrosis with mononuclear cellular infiltration of liver tissues. These histological changes in exposed fish are associated with the response of hepatocytes to pollutants and the type of xenobiotic metabolism in liver tissues. This study demonstrated that copper injected solely is more damaging to the liver than nTiO$_2$ alone and the binary mixture. This might be due to the fact that copper concentration injected alone exceeded the 20 μg/g that can be toxic and harmful to aquatic organisms’ organs [57]. It could also be suggested that the presence of a larger concentration of copper in fish must have not only impaired the liver function and hepatocytes but also triggered the disruption of salt transport such as sodium chloride and potassium chloride into and out, responsible for the homeostatic balance and physiological equilibrium of fish. On the other hand, the presence of nTiO$_2$ either alone or in mixture was mildly harmful than copper. This could be attributed to the fact that nTiO$_2$ which is known to aggregate...
in tissues can also trap other compounds with consequences on their availability. Therefore, this observation could imply the reason for the reduction of damages.

In this study, the effect of Cu, nTiO2 and binary mixture on C. gariepinus liver’s tissues were studied for their biochemical constituents using FTIR technique for analysis. The applicability of this technique is mainly known to assess and monitor the vibrational modes of functional groups. Shift in peak position, changes in bandwidth intensities and band area values of the spectra are mainly used to obtain structural and functional data about the sample analysed [36,45].

The spectra of the control, Cu, nTiO2 and binary mixture of exposed liver tissues of C. gariepinus in the 4000–400 cm−1 region was shown in Fig. 3. The spectra showed several main bands from different functional groups (lipids, protein, nucleic acid and amino acids) (Table 1). The figure demonstrates changes in absorbance intensity between control and treated liver tissues with adverse impact of the compounds on its biochemical constituents.

In the present study spectral analyses were carried out in three main frequencies range as 3600-3050, 3050–2800 and 1800–800 cm−1. Fig. 4 shows the infrared spectra of control and exposed liver tissues in the 3600-3050 cm−1 region. This region is mainly assigned to the OH asymmetric stretching of protein.

This study, the spectra in the region 3050–2800 cm−1 was observed for the control and exposed treated liver tissues in order to investigate the changes in lipids content. In this region, lipids are characterized by CH2 stretching vibrations. Fig. 5 showed defined peaks at 2925 and 2853 cm−1 that are assigned to CH2 asymmetric stretching vibrations of mainly lipids.

Fig. 6 FTIR spectra showed the control and treated liver tissues in the 1800–800 cm−1 region. The sharp peaks on bands observed at 1654 and 1545 were attributed to amide I and II vibrations of structural proteins. Amide I is mainly associated with C=O stretching vibrations. The Amide II band is mainly due to N−H bending and C−H stretching of the polypeptides and protein backbone.

The bands 1458, 1400, 1240 and 1079 cm−1 was observed in control liver tissues. The peak at 1458 cm−1 is assigned to CH2 mode bending of lipids. The band 1400 is representative of symmetric CH3 bending modes of the methyl groups of proteins. However, the band 1240 cm−1 is attributed to Amide III N−H bending. The band 1079 is assigned to Phosphate (PO2−) vibrations.

Copper is an essential micronutrient relevant for the health of aquatic organisms [17]. Its abusive use in agriculture is of concern, since its bioaccumulation in fish may have consequence on the health of aquatic organisms [17]. However, nTiO2 application is on the rise because of their occurrence in many sectors of activities is on the rise [38]. The nanoparticles have unique properties, small size, large surface area with the capacity of adsorption of other chemicals. Both compounds are hypothesized in this study to have biological effects on exposed tissues.

Accumulation of copper, nTiO2 in liver of fish (supplementary 1) in this study was in agreement with findings of [4,6]. However, the availability of copper increased in fish liver exposed to the binary mixture. This could be attributed to the capability of titanium to chelate and adsorb copper in liver tissues.

However, the presence of copper and titanium nanoparticles in liver tissues implies specific mechanisms of action for toxicity. In this study, copper induced to fish was taken by the liver even though; the present study did not focus on the regulation of oxidative stress that could undergo fish (liver) during exposure. It is possible that the hepatic Cu transport proteins such as metallothionein (MT) were responsible for the decrease of copper and titanias concentration in the hepatic tissues compared to the nominal concentration induced. The uptake of Cu by the liver depends on the up-regulation of MT synthesis; MT function detoxifies metals by chelating available free ions in order to reduce their availability [46]. Furthermore the presence of nTiO2 in fish hepatocytes leads to mechanisms of action known as oxidative stress and sometimes apoptosis induced by reactive oxygen species (ROS) [22]. nTiO2 could have disrupted the immune homeostasis of the liver, but also the redox states that could be responsible for interaction between H2O2 and

| S/N | Wavenumber | Vibrational peak assignment |
|-----|------------|----------------------------|
| 1   | 3296       | Amide A N−H stretching     |
| 2   | 2924       | CH2 asymmetric stretching   |
| 3   | 2853.60    | CH2 of lipids symmetric     |
| 4   | 1654.26    | Amide I (protein C=O stretching) |
| 5   | 1545.67    | Protein band Amide II (N−H, C−N) stretch |
| 6   | 1458.36    | CH2 mode bending of lipid   |
| 7   | 1400.79    | Symmetric CH2 bending modes of the methyl groups of proteins. |
| 8   | 1240.58    | Amide III N−H bending      |
| 9   | 1079       | Phosphate (PO2−) vibrations |

Fig. 4. The representative FT-IR spectra of the control, A, B, C exposed to liver tissues of C. gariepinus in the 3600-3050 cm−1 region. The spectra were normalized with respect to amide I band at 3413.59.
$\text{O}_2^-$ creates OH and O$_2$ that damage unsaturated lipids and oxidizing the cell membranes [12]. Therefore, the mechanisms of action of copper and nTiO$_2$ whether individually or in binary mixture could alter the sensitivity of some biomolecules and impact the spectra obtained with FTIR.

The spectra of treated liver of *C. gariepinus* with Cu, nTiO$_2$ and the binary mixture in the 4000-400 cm$^{-1}$ region shows band difference between the control and exposed liver. In this study, the band 3296 in control attributed to Amide A shifted respectively to 3423, 3415 and 3384 due to exposure to Cu, nTiO$_2$ and binary mixture. The peaks shift in this study might account for the variation of intra and intermolecular interactions such as amide hydrogen bonds in liver tissues. The spectra are due to the vibrational modes of various functional groups of the constituents of tissues [35].

The present study showed that there was decrease of absorption intensities (Table 2) of the CH$_2$ asymmetric stretching vibration (2954 cm$^{-1}$) in Cu and nTiO$_2$. However, the binary mixture showed an increase of intensities. The variation of intensity observed in treated fish compared to the control was accompanied with the shift of peak position (2925 cm$^{-1}$). The increase of intensity in binary mixture is attributed to the increase of lipid order and decrease of acyl chain composition.

The band 2853 cm$^{-1}$ observed in the control was attributed to CH$_2$ symmetric stretching belonging mainly to lipids. The shift of band position was recorded in liver exposed Cu and nTiO$_2$ and Cu + nTiO$_2$. However, an increase of intensity was observed in liver of fish exposed to Cu and decreased in fish exposed to nTiO$_2$ and Cu + nTiO$_2$ suggesting that the presence of nTiO$_2$ alone or in a mixture increase the acyl chain composition and also cause the change of the structural nature of lipid.

This study revealed changes in FTIR peak position and intensities in the 1800-800 regions (Fig. 6) due to exposure to Cu, nTiO$_2$ and Cu + nTiO$_2$. This region is squarely related to the amide I, II and III of proteins and C–C stretching of phospholipids.

In this study, the treated fish with Cu, nTiO$_2$ and the binary mixture compared to the control correlates with the change in intensities of bands assigned for Amide I, II and lipids. The band 1545, 1240 and

| Peak | Control | Cu | nTiO$_2$ | Cu + nTiO$_2$ |
|------|---------|----|---------|---------------|
| 1    | 3296.78 | 3423.03 | 3415.22 | 3384.88 |
| 2    | 2924.77 | 2925.26 | 2925.31 | 2925.79 |
| 3    | 2853.47 | 2853.60 | 2853.70 | 2853.85 |
| 4    | 1654.72 | 1654.26 | 1654.70 | 1654.47 |
| 5    | 1545.46 | 1545.67 | 1545.82 | 1545.66 |
| 6    | 1458.75 | 1458.36 | 1458.68 | – |
| 7    | 1400.79 | 1400.79 | 1400.74 | 1405.53 |
| 8    | 1240.58 | 1240.10 | 1240.13 | 1153.44 |
| 9    | 1079.13 | 1081.49 | 1081.62 | 1079.54 |
1458 in the control liver tissues is assigned to the Amide II N–H bending, C–N stretching; Amide III N–H bending vibrations of protein and CH₂ asymmetric bending mainly lipids with little contribution to proteins respectively [49].

Lipsid are very important in the fluidity of membrane thereby affecting the conformation of membrane protein [34]. In this study, there is shift of peak positions and increase of intensities in Cu and nTiO₂ however; an antagonistic increase was observed in binary mixture in peak control 1545 cm⁻¹. At band position 1240 cm⁻¹ in the control, a shift of position was also recorded in treatment with Cu, nTiO₂ and binary mixture. However there was a significant increase of intensities in all treatment compared to the control (Table 3). This suggests that the changes were ascribed to the presence of the compounds in the liver tissues. Probably, the alterations of intramolecular hydrogen bonding of the amide function due to the compounds lead to change in some function groups.

The asymmetric CH₂ bending vibration was observed at band 1458 cm⁻¹ from peptides chains. The peaks intensity variation was recorded in treatment liver tissues compared to the control however a significant decrease of areas or intensities was observed in tissues exposed to Cu and nTiO₂. The binary mixture exposed to the liver tissues contributed to a further decrease of intensities. The alterations observed in this band indicate change in conformation of the side chains from peptide was due to exposures of tissues [32]. At 1079 cm⁻¹ band areas, increased in Cu and nTiO₂ was observed due to the effects of exposure however, a contrary effect was observed in the binary mixture of exposed liver tissues. The binary mixture may have caused an antagonistic response in liver tissues with consequences on the frequency of intensity. The band 1079 cm⁻¹ in control is assigned to phosphate vibrations though, shift of peaks was observed in liver treated tissues.

Amide I band (Fig. 7) in 1700–1600 region is observed after curve fitting analysis for the control, Cu, nTiO₂ and binary mixture of exposed liver tissues of C. gariepinus. The relative changes observed in peaks position and areas are due to the alterations in the compositions of secondary structures and this is commonly used for their determination [23].

The present study revealed the presence of the bands 1687, 1637, 1636 cm⁻¹ attributed to the protein segment with the β sheet structure [5]. The shift of peak position was observed in exposed fish compared to the control; but the protein structure was conserved. These changes of peak positions of second derivatives amide I (Table 5) also indicate the alterations in the composition of secondary structures.

The percentage (75.70%, 65.86% and 56%) of the β sheet structure in exposed liver tissues with Cu, nTiO₂ and binary mixture was significantly higher compared to the control (7.20%). The intensities (areas) of β turns structures decreased significantly in exposed tissues compared to the control. However, the additive effects of both compounds synergistically decreased the β turns structures of liver tissues exposed to binary mixture. This result indicates that the presence of the exposed compounds caused alterations of the proteins structures.

The percentage (13.13%, 24.30% and 36.80%) of a helix structure in exposed liver tissues with Cu, nTiO₂ and binary mixture was enhanced compared to the control (2.83%). The band at 1655 is assigned to a helix structure [5] which intensities increase significantly compared to the control; the changes observed in the protein structure of liver tissues might be due to the effect of contaminant in the liver. The alterations of secondary structures in the existing proteins may be an expression of the formation of new types of proteins.

The ratios of selected band peaks intensities were estimated to evaluate and compared the biotoxicity of control and exposed liver tissues as reported elsewhere by [32].

The ratios of areas of absorption of bands between the CH₂ asymmetric stretch and the CH₂ symmetric stretch (Table 4) for the control, Cu, nTiO₂ and binary mixture exposed liver tissues are 0.30 ± 0.001, 0.20 ± 0.0001, 695 ± 0.00 and 71.24 ± 0.001 respectively. The ratio indicates an insignificant increase (p > 0.05) of exposed liver to nTiO₂ and binary mixture.

The ratios between the peaks intensities bands associated with amide II and Amide I (I₁₅₄₅/I₃₂₉₆) (Table 4) for the control, Cu, nTiO₂ and binary mixture exposed liver tissues are 0.93 ± 0.2, 16 ± 0.9, 1.65 ± 6.92 and 0.38 ± 15 respectively.

The ratio values between the Amide II and Amide A (I₁₃₅₄/I₅₂₉₆) increase significantly (p < 0.05) 0.009 ± 0.0008, 12.15 ± 9, 17.67 ± 15 and 0.045 ± 0.017 in Cu, nTiO₂ and binary mixture. However, the mixture showed a synergistic decrease in the ratio between Amide II and Amide A.

The above results indicate that exposure induce alteration on the major biochemical component, similar to the study of [32].

4. Conclusion

The present study shows alterations on the major biochemical component and histological pathology indicating the bio-toxic effects of copper, nTiO₂ and the binary mixture. The results of this study demonstrate changes in structural conformation of protein of exposed fish. The amide I bands in this study after curve fitting analysis revealed the presence of dominated structures such as β sheet, β turns and α helix. However β sheet peaks intensity were highly represented in exposed tissues. The increase of β sheet and α helix was observed in exposed fish compared to the control however, the β turns was further decrease due to the additive effect of Cu on nTiO₂. The frequent alteration of secondary structure is an indication that, the structural change of protein conformation stem from the toxicity of the compounds used. However, further study should be conducted to assess the...
Fig. 7. The underlying Amide I bands in the 1700-1600 cm\(^{-1}\) region deduced by curved-fitting analysis for control (A), Cu (B), nTiO\(_2\) (C) and Cu + nTiO\(_2\) (D) of exposed liver tissues of \(C.\) gariepinus.

Table 4

| Band ratio     | Control         | Cu              | nTiO\(_2\)       | Cu + nTiO\(_2\)  |
|----------------|-----------------|-----------------|-----------------|-----------------|
| \(I_{1545/3296}\) | 0.009 ± 0.0008  | 12.15 ± 9.0\*   | 17.67 ± 1.5\*   | 0.045 ± 0.07    |
| \(I_{1545/1654}\) | 0.93 ± 0.200    | 1.6 ± 0.9\*     | 1.65 ± 6.92\*   | 0.38 ± 15       |
| \(I_{2924/2853}\) | 0.30 ± 0.001    | 0.20 ± 0.0001   | 69.5 ± 0.00\*   | 71.24 ± 0.001\* |

Values are shown as means ± standard deviation (n = 5), Significance was denoted as for p < 0.05.
* Indicates significant differences from the control group within treatment. Significance was denoted as for p < 0.05.

Table 5

| Peaks   | Control       | Cu            | nTiO\(_2\)       | Cu + nTiO\(_2\)  | Structure |
|---------|---------------|---------------|-----------------|-----------------|-----------|
| 1687    | 0.256 ± 0.19  | 1.54 ± 7.97\* | 1.81 ± 5.86\*   | 1.21 ± 1.68\*   | \(\beta\) Sheet |
| 1666    | 16.25 ± 63.23 | 5.90 ± 10.97\*| 3.97 ± 10.51\* | 1.71 ± 2.72\*   | \(\beta\) turns |
| 1655    | 0.874 ± 0.37  | 5.02 ± 11.35\*| 9.80 ± 31.93\*  | 8.77 ± 28.98\*  | \(\alpha\) Helix |
| 1637    | 0.072 ± 0.021 | 0.03 ± 0.03\*  | 12.59 ± 63.37\* | 0.14 ± 0.05\*   | \(\beta\) Sheet |
| 1636    | 13.27 ± 394.59| 17.10 ± 33.41\*| 12.16 ± 151.25\*| 6.21 ± 49.89\*  | \(\beta\) Sheet |

Values are shown as means ± standard deviation (n = 5), downward arrow indicates a decrease and upward an increase compared to the control. \* indicates significant differences from the control group within treatment. Significance was denoted as for p < 0.05.
mechanisms pathway of the binary mixtures.

Author’s contribution

The author conceived, analyzed, wrote and prepared the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version, at doi:https://doi.org/10.1016/j.toxrep.2019.10.002.

Table 6

| Structure | Control | Cu | nTiO₂ | Cu + nTiO₂ |
|-----------|---------|----|------|-----------|
| β Sheet (%) | 44.46 | 75.70 | 65.86 | 56 |
| β turns (%) | 52.71 | 11.17 | 9.84 | 7.20 |
| α Helix (%) | 2.83 | 13.13 | 24.30 | 36.80 |

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