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EFFECTS OF TITANIUM DIOXIDE NANOPARTICLES EXPOSURE ON THE VITAL ORGAN OF CTENOPHARYNGODON IDELLA

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Abstract

The aim of the current study was to analyze the histopathological changes in the organs of Ctenopharyngodon Idella. Titanium dioxide nanoparticles (TiO2 NPs) were widely produced and released into the environment, posing a serious threat to fish and human health. Titanium dioxide nanoparticles (Np-TiO2) have become the common component of sunscreen cosmetic products. Np-TiO2 can affect especially aquatic ecosystems' health, including aquatic organisms such as fish. It is therefore necessary to acquire a better understanding of the effect of Np-TiO2 on aquatic organisms. This study evaluated the biological effects of Np-TiO2 on grass carp, such as survival rate and weight change and, in particular, the Ti content or retention in the gill, heart intestine, and liver. In addition, the structure of the intestine, gill, heart, and liver was investigated through histological analysis. Three hundred grass carp fish were used, randomly divided into five treatment groups: a control group (fed with food without adding Np-TiO2) and Titanium dioxide nanoparticles at different concentrations (0.5 mg/kg,1 mg/kg, 1.5 mg/kg, 2 mg/kg) were incorporated with a fish diet during an 8-week period. The amount of Ti in the gills, liver, heart, and intestine was measured using atomic absorption spectrophotometry coupled to a graphite furnace (GFAAS). After treatment of titanium dioxide nanoparticle and histopathological analysis organ showed gill hyperplasia, necrosis, an increase of mucous secretion, mild hyperplasia, and a reduction in gill filament. In the heart dispersion and swelling, Necrosis, and Erosion were reported. In the liver cloudy swelling, necrosis, mild acute inflammation, and hydropic degeneration were reported. In the intestine were signs of necrosis, chronic non-specific inflammation, an increase in the number and size of goblet cells, and an increase in the number of lymphocytes. In conclusion, the presence of TiO2-NPs in natural water bodies may affect the aquaculture industry as well as inland fisheries.

INTRODUCTION

Nanoparticles refer to substances that consist of less than 100 nanometres. Nanoparticles consist of size with physical and chemical properties different from materials that are usually in the environment (Khan

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et al., 2022). There are many other kinds of nanoparticles that consist of different factors. Some are natural ones that are founded in soils, natural water, or the dust of volcanos. (Strambeanu et al., 2014) Nanoparticles are able to be produced through biological as well as geological processes. Many organisms when even toxic can adapt and evolve in rich environments as nanoparticles. (Zhang et al., 2018) They are produced by both geological and biological processes. Even when toxic, many organisms can adapt and evolve in an environment rich in natural NPs (Mannoush et al., 2022).

Nanoparticles are capable of passing through the blood –barrier, and blood-eye barrier. They can cause various xenobiotic effects. Nanoparticles can be toxic to the environment when dumped carelessly. (Tabassum et al., 2020) Because of their small size, nanoparticles have a large surface area as compared to their weight. This provides a surface medium for reaction and leads to the adherence of toxic materials to nanoparticles (Khan et al., 2019). Nanomaterial can come about as expected originally, and be shaped as the by-foods of burning reactions may be produced decisively through engineering to perform some of the specialized roles.(Shaban et al., 2024) Due to their capability of generating materials in a specific way to show an unambiguous function, the use of nanomaterials spans a wide range of engineering fields, from healthcare to cosmetics, preservation, and air purification (Qaiser et al., 2018). For example, in the healthcare field, many used nanomaterials are occurring in various ways, with the main use of drug delivery. (Khan et al., 2022) One of the illustrations of this method is whereby these (Jia et al., 2023). Nanoparticles are being industrialized to assist the transference of chemotherapy medications openly to cancerous growth cells and deliver treatments to sites of arteries that are being damaged to fight cardiovascular disease. (Pala et al., 2020)

Titanium dioxide nanoparticles (TiO2-NPs) are rapidly increasing widely due to refraction high index and luster which involves the white appearance of TiO2-NPs (Markowska Szczupak et al., 2011). TiO2-NPs are one of the top five types of NPs manufactured and used in the world with an annual production of above 10,000 metric tons, which is expected to reach 2.5 million tons by 2025 (Hajirezaee et al., 2020). TiO2-NPs are also greatly used as an efficient water-treatment agent in the decomposition of organic impurities and chemicals present in the water through their catalytic activity (Ojha and Nemiwal, 2022).

Between 1916 and 2011, the world produced 165,050,000 metric tonnes of titanium dioxide (TiO2) pigment (both nano and bulk) (Suresh, 2021). As per the report published by Global Market, the Titanium Dioxide Market size was predictable at $17 billion in 2019 and is expected to hit $27.2 billion by 2026, registering a CAGR of 7.7% from 2020 to 2026. Nano-TiO2 is used in a variety of products, including personal care, household goods, and food. The human consumption of nano-TiO2 as a food product is predicted to be 1 mg kg 1 body weight per day (Li et al, 2023).

They are developed by the process including biological and geological processes. Even when toxic, many organisms can acclimate and develop in an environment rich in natural NPs (Mannoush et al., 2022). Nanomaterial can come about as expected originally, and be shaped as the by-foods of burning reactions may be produced decisively through engineering to perform some of the specialized roles. Due to the capability of generating the materials in a specific way to show an unambiguous function, the use of Nanomaterial areas across an extensive variety of engineering, starting from healthcare and another kind of cosmetics products to many preservations and also used in air purification. For example, in the field of healthcare, many used nanomaterials are occurring in a variety of ways, with the main use of drug delivery. (Jia et al., 2023) Nanoparticles are being industrialized to assist the transference of chemotherapy medications openly to cancerous growth cells, as well as deliver treatments to sites of arteries that are being damaged to fight cardiovascular disease. (Yang et al., 2023)

Titanium compounds are used in conditions where insubstantial strength and capability to endure temperature prodigality are required. Titanium may also be used in the salt removal process of plants. The metal is often used for mechanisms that somehow are exposed to salty or seawater (Bah et al., 2022). A titanium anode dusted with platinum may also be used to offer a cathodic- corrosion shield

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from salty and seawater. Because it is inverted in anybody, titanium metal has many surgical applications. Titanium oxide solutions hold account for the biggest use of the component. Titanium oxide is also used in some cosmetics to dissolve light. Since this compound exhausts strongly in the air, it can also use to produce different smoke screens (Markowska, et al 2020).

This study aimed to evaluate the biological effects of Np-TiO2 on grass carp because Ctenopharyngodon Idella is a widely distributed native Chinese freshwater fish. For aquatic weed control, these kinds of species are served in 50 countries or more countries across the world. Grass carp play a vital role in human consumption as the source of protein in many countries and are an essential part of the fish culture (Lin et al., 2022). It is the world's third most commonly cultured species due to its success (Welima, 2004). Grass carp is one of China's most predominant freshwater fish, with an annual production of about 4 × 106 tons in 2009, but it has not been developed into a high-value product (Long et al., 2023; Hu et al., 2021). Most importantly the study determined the Np-TO2 content of the gill, heart, intestine, and liver of the experimental fish. Furthermore, the structure of the intestine, gills, heart, and liver were investigated through histological analysis. The present work also sought to provide more nuanced insight into the histology of these organs.

Material and methods

Ethical statement

This work was approved by the animal research ethics committee and carried out in accordance with the instructions for the care and treatment of laboratory animals at the University of Lahore Sargodha campus.

Ctenopharyngodon Idella

Grass carp fingerlings have an average initial weight of 18. 8 ± 0.15 g and an initial length of 14.2±0.11 were purchased from the fish seed hatchery and acclimated to laboratory conditions (ABNT, 2007) for at least one week before the start of the experiment. The fish were then distributed into five treatments three replicates per treatment, of tweeting fish per treatment, and each treatment was conducted over a 14-hour light/ten-hour dark cycle in glass aquaria (70 L) with filtered water maintained at 26 C, pH 6.8, and 5.0 ±0.05 mg of oxygen content. Over the acclimation and trial periods, commercial feed from AMG Pvt. Ltd which included 22% protein was supplied twice daily to the fish in the five treatments.

Experimental Design

The present study intended to introduce TiO2 -Np to the experimental fish via food. Following acclimation of the five treatments three replicates per treatment of 20 fish each were randomly designated into treatments, T1, T2, T3, and T4, with the Control group designated as fish receiving food without Np-TiO2, T1 with 0.5mg/kg of TiO2-NPs, T2 with 1mg/kg of TiO2-NPs, T3 with 1.5mg/kg of TiO2-NPs and T4 with 2mg/kg of TiO2-NPs.

Food preparation

The food was prepared from a commercially available fish diet (Alcon Basic), without or with Np-TiO2 (Eusolex T-aqua, 30% of TiO2-Np Merck, USA) added to it according to desired concentration following Fouqueray and coworkers (2012). Thus, the control group received only commercial food while T1, T2, T3, and T4 received commercial food with the desired TiO2- Np amounts, that is, T1 received 0.5 mg/kg of TiO2 -Np for each 1 g of food, T2 received 1mg/kg of TiO2 -Np for each 1 g of food, T3 received 1.5mg/kg of Np-TiO2 for each 1g and T4 received 2mg/kg of Np-TiO2 for each 1 g of food. Fish were fed with commercially available fish food at a rate of 3% body weight per day. Fish were fed for fifteen days and a 24-h fast. Eight fish were removed from each group, weighted individually, and on the fifteenth day following the Brazilian Federal Council of Veterinary Medicine (Law No. 5.517/68 article 16, "f")

Histological analysis

Histological observations were performed as described in Mansouri et al 2016. On the 60th day, fish were dissected for histology. Finally, the fish tissues were carefully removed and fixed in a formalin solution. Then, the tissues were dehydrated using a series of graded ethanol solutions, cleared in xylene, and embedded in paraffin wax, and a section

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of 5um was prepared from paraffin blocks using a rotary microtome. These sections were then stained with hematoxylin –Eosin and examined microscopically. Moreover, the histopathological abnormalities were evaluated using a modified version of the protocol described by Bernet et al. 1999.

Results:

Histopathology effects in the gill

Mucus secretion and aggregates on the outer surface of grass carp are observable. This condition in some fish of different treatments was detected with increasing concentrations of nanoparticles and a number of days. The histological anomalies of fish gills exposed to a different type of TiO2 nanoparticles. In the control group, secondary lamellae length and diameter and mean of the gill filament diameters consist of only some small histopathological alterations. According to the results of this study, some histopathological lesions in the gill of grass carp such as hyperplasia, curvature, an increase of mucous secretion, necrosis, and reduction in diameter of gill filaments were observed. Initial damage of NPs on grass carp gill contains dilated and clubbed tips and curvature of the lamella. Moreover, the hyperplasia and fusion of the gill filament were visible in most treatments. The changes in diameter and length of gill filaments exposed to different types of nanoparticles. In Ctenopharyngodon Idella the toxic effect of nanoparticles was dominant in gill with an increase in concentrations.

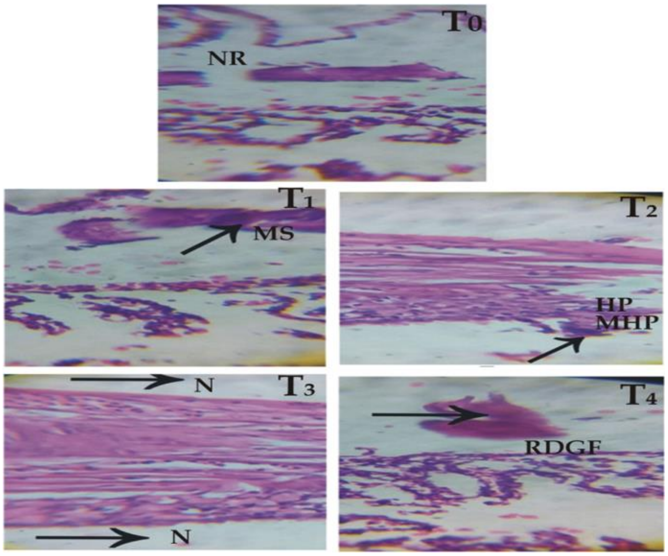

Figure 1: Gill morphology in grass carp. The gills of the control fish show normal arraignment (NR) of tissue, whilst all treatments (T1, T2, T3, T4) showed injuries that include mucous secretion (Ms), hyperplasia (Hp), mild hyperplasia (MHp), necrosis (N), reduction in diameter of gill filaments (RDGF).

Histopathology effects in the intestine

The histological changes of intestine tissue observed in the different types of TiO2-NPs exposed and controlled fish are seen in Figure. The results of this study indicated that the most common changes in the intestine are degeneration, chronic non-specific inflammation, lymphocytic inflammation, an increase in the number of lymphocytes, and necrosis. In the case of the intestine tissue samples, the frequency of abnormalities was higher in the TiO2 NPs intestine samples when compared with the control samples. According to the study necrosis and intensity of inflammation were more dominant in a higher concentration of titanium dioxide nanoparticles. Necrosis and an increase in the number of lymphocytes were most commonly observed in the intestine of Ctenopharyngodon Idella. Microscopic studies of these were observed in each case. The effects of the TiO2 -NPs were shown in Figures.

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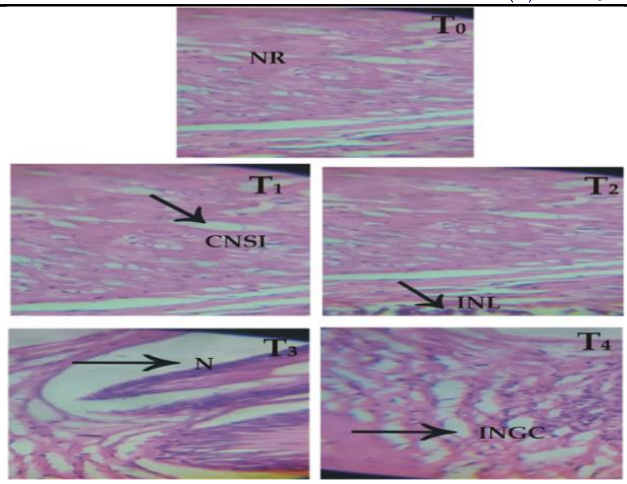

Figure 2: Intestine morphology in grass carp. The intestine of the control fish shows normal arraignment (NR) of tissue, whilst all treatments (T1, T2, T3, T4) showed injuries that include chronic non-specific inflammation (CNSI), an increase in the number of lymphocytes (INL), Necrosis (N), increase in the number of globet cells (INGC).

HISTOPATHOLOGICAL EFFECT ON THE LIVER:

The histological changes of liver tissue observed in the different types of TiO2-NPs exposed and controlled fish have seen in Figure. Different concentrations of titanium dioxide nanoparticles showed histological effects on the Liver. The Most common types of effects were Cloudy swelling, necrosis, mild acute inflammation, and Hydropic degeneration. Higher concentration of titanium dioxide nanoparticles Cloudy swelling and Hydropic degeneration in the liver of Ctenopharyngodon Idella.

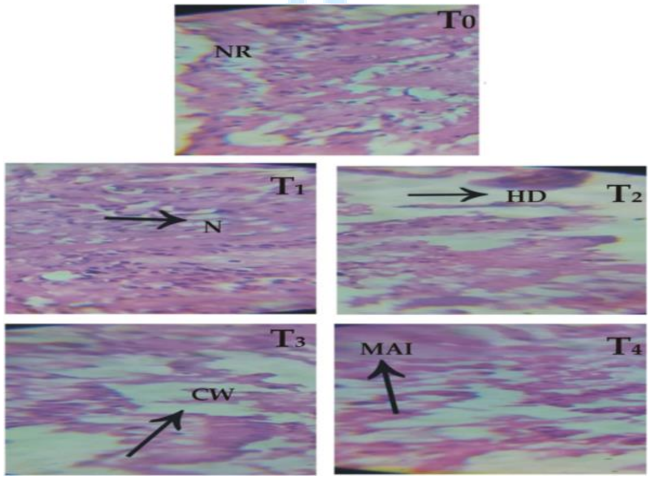

Figure 3: Liver morphology in grass carp. The liver of the control fish shows normal arrangement (NR) of tissue, whilst all treatments (T1, T2, T3, T4) showed injuries that include Necrosis (N), hydropic degeneration (HD), Cloudy swelling (CW), and Mild acute inflammation (MAI).

HISTOPATHOLOGICAL EFFECT ON THE HEART:

The histological change of heart tissue observed in the different types of NPs exposed and controlled fish have seen in fig. The results of this study indicated that the most common changes in the heart are necrosis and erosion, dispersion, and swelling. The Heart shows abrasion when exposed to different concentrations of titanium dioxide nanoparticles. The highest concentration of

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nanoparticles caused damage to heart tissues. When we compare the effects of observation all the tissue and organ shows abrasion in their structure. The highest concentration shows the most dominant effect in different studied parts as visualized under a microscope.

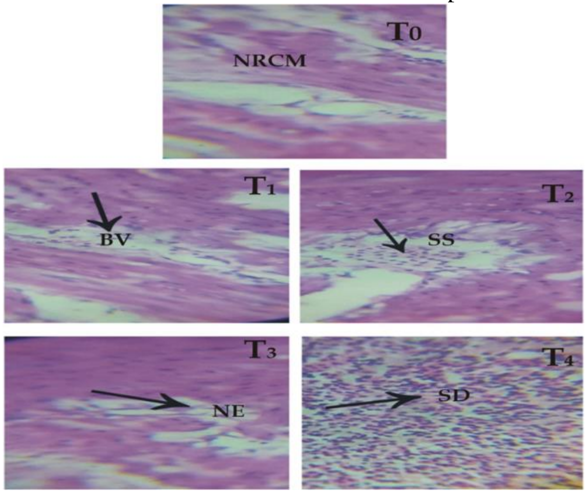

Figure 4: Heart morphology in grass carp. The heart of the control fish shows normal arraignment (NR) of cardiac muscle, whilst all treatments (T1, T2, T3, T4) showed injuries that include blood vessel (BV), Signs of Swelling, Necrosis, and Erosion (NE), Signs of dispersion (SD).

Discussion:

Due to the increasing use of nanomaterials, nanoparticles containing Ti can gain entry into the aquatic environment in large amounts. Most studies that assess the effect of Np-TiO2 on fish expose them to water contaminated experimentally with Np-TiO2 (Hund-Rinke and Simon, 2006; Chen et al., 2011). Chen et al. (2011), for instance, provided proof that only gills displayed histopathological alterations in fish exposed to Np-TiO2. On the other hand, mild to moderate histopathological alterations occurred in the gills and liver of Oreochromis niloticus exposed to it for 72 h (Ramsden et al., 2009).

The current study presents the effects of titanium dioxide nanoparticles in Ctenopharyngodon Idella concerning histology. Gill, in fish, is among the most important organs in a fish's body and performs several critical functions in the body including body fluid permeability balance, osmoregulation, and respiratory gas exchange (Jayaseelan et al., 2014). It is also the main target organ for nanoparticle toxicity because it produces harmful consequences (Griffitt et al. 2007). Because of its large surface epithelium area and direct contact with water, the gill organ is more vulnerable to chemical pollutant impacts in aquatic systems (Maleki et al., 2015). The most common histopathological abnormalities in fish gills identified in this study are hyperplasia, edema, curvature, shortening, and fusion of gill lamellae, aneurism, and necrosis are among the most prevalent histological abnormalities in fish gills identified in this study. These histological changes indicate that nanoparticles are affecting the gills and other fish species exposed to nanoparticles have suffered similar effects (Song et al., 2015), like titanium dioxide nanoparticles (Federici et al. 2007), So here in the present study as visualized under microscope stated that higher concentration of nanoparticle affected the gills most as confirm in the study.

Another frequent change in the gills of fish exposed to Nanoparticles is edema. This shift might be due to the acute inflammatory character of the lesions that are generated in such gills, and gill edema could be caused by epithelial sodium pump failure (Mansouri et al., 2017). The distance between the pollutant and the circulation is increased by epithelial edema, whereas fusion lowers the gill surface and therefore the interaction between the pollutant and the gill epithelium (Bhuvaneshwari et al., 205), which are thought to be a combination of nanoparticle aggregation and mucus discharge from fish gills, demonstrate that these Nanoparticles might enter the fish body straight via the damaged epithelial cell membrane and cause the fish to die. Furthermore, as

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the lamellar epithelium decreases and fuses, gas and ion exchange between the lamellae decreases, resulting in unfavorable side effects, which have an impact on fish health (Jiraungkoorskul et al., 2002).

Furthermore, these findings show that the severity of damage to the fish gills rises as the concentration of Nanoparticles increases, but that the severity caused by Nanoparticles and titanium dioxide Nanoparticles is greater than that caused by titanium dioxide Nanoparticles alone and the control group. Aneurism is a blood-filled, expanding blood artery in the gill tissue that can cause disruptions in blood flow, rupture, severe hemorrhage, and bleeding, as well as death (Rajkumar et al., 2012). Circulatory abnormalities, such as aneurism, regressive and progressive alterations, and hyperplasia, are the most common causes of histological reactions in fish gills (Van Dyk et al. (2009). Similar aneurism lesions in the gills of Danio rerio were reported by Mansouri et al. (2015) after eight days of exposure to nanoparticles and cobalt ions at concentrations of 10, 40, 70, and 100mg in treatment 1. In addition, Al-bairuty et al. (2013) indicate that 10 days of exposure to 20 and 100 mg treatment 1 of copper nanoparticle causes some increases in the rate of disease.

According to Zou et al. (2014), the presence of titanium dioxide Nanoparticles (1.5mg in treatment 1) in various lighting modes alters the surface chemistry of silver nanoparticles (1.5 mg treatment 1), resulting in distinct toxicity effects in the ciliated protozoa Tetrahymena pyriformis after a 24-hour exposure period. They then claim that Nanoparticles lower the environmental hazards of silver nanoparticles in natural light, while titanium dioxide nanoparticles increase the environmental risks of silver nanoparticles in continuous light.

Zhang et al. (2013) demonstrated that the toxic effects of 0.1, 10, and 100 mg treatment 1 in zebrafish in the presence of 6 mg treatment 1 dots are closely dependent and of greater toxicity with increasing dose, and that the joint toxicities of the two toxicants are synergistic for 6 to 120 h of exposure. Furthermore, Shi et al. (2010) indicate that simultaneous exposure to trace Nanoparticles (0.1 g) induces dose-dependent synergistic Genotoxicity, implying a possible environmental danger of assisted photocatalysis for 36 hours. Because the present concentration of titanium dioxide nanoparticles had a greater impact on the body than other concentrations.

In this investigation, swelling and an increase in the number of goblet cells were observed in the gut of common carp, indicating a defense mechanism against severe pathogenic alterations. Goblet cells secretions, on the other hand, are considered to offer protection for the epithelium as well as lubrication to aid food transit, this was according to Carlson et al. (2006). Goblet cells produce mucous substances that may protect the epithelium throughout the digestive system by promoting food passage, according to Facial et al. (2015). On the other hand, the gut, as one of the body's essential organs that are injured by contaminated food, is also affected by the touch or consumption of polluted water. Water polluted with titanium dioxide nanoparticles for up to 14 days causes significant damage to the epithelial tissue of the gut of rainbow trout O. my kiss, according to Federica et al. (2007). Ingestion of nanoparticle-contaminated water, as well as the direct impacts of nanoparticles on gastrointestinal tract tissue, via endocytosis, may affect the absorption of these chemicals by the internal organs (Handy et al., 2008). In present study stated that certain organs are affected by the increase in the concentration of titanium dioxide nanoparticles.

CONCLUSION:

In the current study, our data supported the effect of titanium dioxide nanoparticles on Ctenopharyngodon Idella on vital organs. After treatment of titanium dioxide nanoparticle histopathological analysis organ showed gill hyperplasia, necrosis, mild hyperplasia, and reduction in gill filament. In the liver, necrosis, cloudy swelling, mild acute inflammation, and hydropic degeneration were reported. In the intestine were signs of necrosis, chronic non-specific inflammation, lymphocytic inflammation, and an increase in the number of lymphocytes.

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