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Titanium dioxide nanoparticles induce energy perturbation by stimulating glycolytic metabolic profile in rats

Samuel Kehinde[1,*], Oluwatobi Adelesi[2], Regina Ngozi Ugbaja[2], Enock Dare[3] and Oladipo Ademuyiwa[2,*]

[1]Department of Environmental Health Science, Faculty of Basic Medical Sciences, Ajayi Crowther University, Oyo, Nigeria.
[2]Department of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria.
[3]Department of Chemistry, College of Physical sciences, Federal University of Agriculture, Abeokuta, Nigeria.

***Correspondence:** samuelkehinde0707@gmail.com and adelad2@yahoo.com.

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ABSTRACT

The safety of titanium dioxide nanoparticles (TiO_2-NPs) remains uncertain due to a scarcity of data regarding its absorption, distribution, elimination, and potential adverse effects following oral exposure. As emerging evidence suggests perturbations in energy metabolism play a pivotal role in the toxicity induced by various toxicants, this investigation aimed to assess the effect of TiO_2-NPs on the activities of specific glycolytic enzymes in rats. In this study, seventy-two (72) male Wistar rats (200 ± 20g) were divided into 12 groups, each consisting of 6 animals. The rats were orally exposed to TiO_2-NPs (8-12nm) at doses of 50, 150, and 250 mg/kg body weight (BW) for durations of 4, 8, and 12 weeks. The control groups received distilled water. The results showed that, except for the 150 mg/kg BW dose of 12 weeks, TiO2-NPs exposure led to an up-regulation in hexokinase activity in lymphocytes, plasma, erythrocytes, and the liver. Hepatic and lymphocyte aldolase activities were also up-regulated, except at 8 and 12 weeks for the 50 and 150 mg/kg BW doses, where slight decreases were observed. Plasma aldolase increased, except at 12 weeks for 150 mg/kg BW dose, while erythrocyte aldolase increased only during the 4 and 8-week exposure but decreased throughout the 12-week exposure. Notably, compared to erythrocyte lactate dehydrogenase activity, which decreased, a consistent pattern observed in lymphocytes, plasma, and hepatic enzymes was a down-regulation at 8 and 12 weeks for the 50 and 150 mg/kg BW doses of TiO_2-NPs. In other instances, and time intervals, lactate dehydrogenase was up-regulated. The data from this study underscores that exposure to TiO_2-NPs can disrupt the glycolytic pathway of energy metabolism, particularly during the initial 4 weeks of exposure. These perturbations are characterized by increased glycolytic enzymes activity in the lymphocytes, plasma, and the liver.

Keywords: *Titanium dioxide nanoparticles; energy metabolism; glycolytic; lymphocytes and hexokinase*

INTRODUCTION

Objects with at least one of their three dimensions lying within the range of 1 to 100 nanometers are referred to as nanoparticles (Joudeh & Linke, 2022). When compared to bulk materials like powders, plates, and sheets, these particles have an extraordinarily high surface area-to-volume ratio and are very small to be observed by the human eye (Khan et al., 2022). In addition, due to their relative size, which allows them to confine their electrons and peoduce

quantum effects, nanoparticles have unexpected physical, optical, chemical characteristics. Nanomaterials find importance in a wide variety of industries, from medical and cosmetics to preservation of environment and purification of water and air, thanks to their ability to be designed for specific objectives (Naseem & Durrani, 2021).

With a molecular weight of 79.9 g/mol, titanium dioxide (TiO_2) is an inflammable, odorless and white powder. At 25 °C, it has a boiling point of 2972 °C, relative density of 4.26 g/cm3 and melting point of 1843 °C. TiO_2, a hardly soluble particle, has find wide array of use as a white pigment (Shi et al., 2013). TiO_2 particles with a diameter of less than 100 nm are referred to as ultrafine titanium dioxide nanoparticles. TiO_2 has two crystal structures: rutile and anatase, the latter of which is more chemically reactive. According to certain studies, TiO_2 in its anatase form may be more harmful than in its rutile form (Zerjv et al., 2022).

Due to their improved catalytic activity compared to TiO_2 in its normal form, TiO_2 nanoparticles are now used in a wider range of industrial, consumer, and medicinal applications. This is a result of the development of nanotechnology. One of the most produced nanomaterials is TiO_2 NPs along with silicon dioxide and zinc oxide particles. It's the second-most-commonly used nanomaterial in consumer goods after silver nanoparticles (Mustapha et al., 2020). TiO_2 might be referred to as a "legacy nanomaterial" due to its vast historical use as an indispensable chemical.

Due to its potential to block UV light while retaining transparency on the skin, TiO_2-NPs finds use in a variety of industries, including sunscreens. For its ultraviolet absorption and photocatalytic sterilizing qualities, which are employed in anti-fog coatings and self-cleaning windows, it is also utilized in the construction industry as an additive to paints, plastics, cements, windows, tiles, and other items. Light-emitting diodes and solar cells can benefit from the use of engineered TiO_2 nanoparticles. Additionally, organic compounds in wastewater can be broken down using TiO_2's photocatalytic activity. To customize them for particular uses, TiO_2 nanoparticle products are occasionally coated with silica or alumina, or doped with other metals (Rabajczyk et al, 2021).

Researchers are concerned about the impeding adverse effects of TiO_2-NPs on human health due to their reduced dimensions since as particles get smaller, their surface areas proportionally grow (Rabajczyk et al, 2021). The unique bioactivity and health risks posed by TiO_2-NPs have come under scrutiny due to this size reduction (Baranowska-Wójcik et al., 2022)

Numerous studies have pointed to the toxicity of TiO_2-NPs, particularly concerning various routes of exposure (Pujalté et al., 2017; Lama et al., 2020; Brand et al., 2020; Horník et al., 2021; Driscoll, 2022; Baranowska-Wójcik et al., 2022). However, the safety of TiO_2-NPs remains uncertain, primarily because of limited data on their toxicokinetics and potential negative effects following oral route of exposure. Additionally, there is a growing awareness that disruptions in energy metabolism through the glycolytic pathway play a critical role in the toxicity induced by toxic substances. It has been demonstrated that TiO_2-NPs can be absorbed through the gastrointestinal tract and distributed to other organs (Jo et al., 2015; Putra et al., 2018; Gubatan et al., 2023). Therefore, there is a pressing need to comprehensively investigate the impact of oral exposure to TiO_2-NPs on glycolytic energy metabolism. This research aims to elucidate the time-dependent and dose-dependent effects of TiO_2-NPs exposure on glycolytic energy metabolism in male Wistar rats.

METHODOLOGY

Chemicals

Titanium dioxide nanoparticles (8-12nm) were procured from Carlroth GmBH, located in Karlsruhe, Germany. Diagnostic kits required for the assessment of total protein and lactate dehydrogenase activity were sourced from CYPRESS® Diagnostics, Belgium. All other chemicals used were of the highest purity and analytical grade.

Animals and treatment

Seventy-two- healthy male Wistar rats, with weights ranging from 180 to 220 grams, were obtained from the College of Veterinary Medicine at Federal University of Agriculture, Abeokuta, Nigeria. These animals were accommodated in the animal housing facility and provided with a standard pellet diet and maintained under controlled temperature of 22±2°C and a 12-hour light-dark cycle. The rats were randomly divided into twelve groups, each comprising six animals. Among these groups, three were subjected to oral exposure of TiO_2 NPs (50, 150, and 250 mg/kg BW) for 4, 8, and 12 weeks, respectively while three control groups receiving distilled water during the same duration. International standards for the use and care of experimental animals were followed in handling the animals (NRC, 2011). Following an overnight fast, blood samples from the rats were taken via the abdominal artery while they were being lightly sedated after the TiO_2NPs exposure time was complete. Liver tissues were also excised for subsequent biochemical analyses.

Preparation of lymphocytes, blood plasma and erythrocytes

Blood samples were processed by centrifuging the blood to obtain the plasma and erythrocytes, while lymphocytes were also isolated by differential centrifugation method described by Boyum (1976). Subsequently, all samples were carefully preserved at -20°C.

Assay of glycolytic enzymes

Glycolytic enzymes activity, including hexokinase, aldolase, lactate dehydrogenase, and NADase, were assayed for in erythrocytes, lymphocytes, plasma, and liver tissues.

Determination of hexokinase activity in the erythrocytes, lymphocytes and plasma.

The determination of activity of hexokinase was carried out in accordance with the protocol outlined by Colowick (1973). This assay uses a coupled reaction of glucose-6-phosphate dehydrogenase (G6PD) and NAD^+ to reduce NAD^+, and spectrophotometrically measured by observing the increase in absorbance at 340 nm.

Determination of the hepatic hexokinase activity

To obtain a 10% liver homogenate, 0.2 g of liver tissue were homogenized in 1.8 mL of cold hexokinase assay buffer comprising 0.05M Tris-HCl buffer (pH 8.0) to achieve a 10% homogenate. Subsequently, after centrifuging the homogenate (5000 rpm, 10 minutes, 4°C), the supernatant was cautiously transferred to Eppendorf tubes that were previously cooled. An aliquot of the supernatant was used for the hexokinase assay, following the protocol described by Colowick (1973).

Determination of aldolase activity in the erythrocytes, lymphocytes and plasma

Aldolase activity was measured according to the procedure described by Jagannathan et al. (1956), which makes use of Boyer's modification of the hydrazine assay. In this test, 3-phosphoglyceraldehyde and hydrazine react to produce a hydrazone that has an absorbance peak at 240 nm.

Determination of hepatic aldolase activity

To obtain a 10% liver homogenate, 0.2 grams of liver tissue were homogenized in 1.8 mL of cold aldolase assay buffer (containing EDTA 0.001M and hydrazine sulfate 0.0035M, pH 7.5). The resulting homogenate was subsequently centrifuged (5000 rpm, 10 minutes, 4°C). The obtained supernatant was carefully transferred to pre-cooled Eppendorf tubes and used for the aldolase assay as described by Jagannathan et al. (1956).

Determination of the activity of LDH in the erythrocytes, lymphocytes and plasma

LDH facilitates conversion of pyruvate using NADH, and the speed at which NADH concentration diminishes, as detected spectrophotometrically at 340nm, is directly linked to the concentration of catalytically active LDH. Utilizing the LDH Kit and following the manufacturer's instructions, the activity of LDH was evaluated.

Determination of hepatic LDH activity

A 10% liver homogenate, obtained by homogenizing 0.2 grams of liver tissue in 1.8 mL of pre-cooled LDH assay buffer (0.2M Tris-HCl buffer; pH 7.3). The homogenate underwent centrifugation (5000 rpm; 10 minutes; 4°C). The resulting supernatant was transferred to pre-cooled Eppendorf tubes. A portion of this supernatant was subsequently used for the lactate dehydrogenase assay.

Determination of NADase activity in the erythrocytes, lymphocytes and plasma

This was determined with the method outlined by Kaplan et al. (1995). This assay involves the reaction of cyanide with the quaternary nitrogen form of NAD^+ to generate a product, which exhibits its highest absorbance at 340 nm.

Determination of hepatic NADase activity

To obtain a 10% liver homogenate, 0.2 grams of hepatic tissue were homogenized in 1.8 mL pre-cooled NADase assay buffer (0.1M potassium phosphate buffer; pH 7.5). The resulting homogenate was centrifuged (5000 rpm, 10 minutes, 4°C), and the supernatant was carefully transferred to pre-cooled Eppendorf tubes which was subsequently utilized for the NADase assay, following the protocol of Kaplan et al. (1956).

Statistical analysis

The results are shown as mean ± SEM. One-way analysis of variance (ANOVA), followed by the Tukey's test was used to analyse the findings, with a significance level of $p < 0.05$.

RESULTS

TiO_2-NPs modulates hexokinase activities in Wistar rats.

Hexokinase activity in the lymphocytes, plasma, erythrocytes, and liver of rats exposed to TiO_2-NPs is presented in Figure 1. Following 4 weeks of exposure, TiO_2 NPs led to an increase in lymphocyte hexokinase activity, with a respective fold change of 3, 3, and 2 at 50, 150, and 250 mg/kg BW. Conversely, significant decreases (59%) were observed after 8 weeks at 50 mg/kg BW and 12 weeks at 150 mg/kg BW (66%). Notably, the most substantial increase in lymphocyte hexokinase activity occurred at 4 weeks, following a hormetic pattern in comparison to the control group.

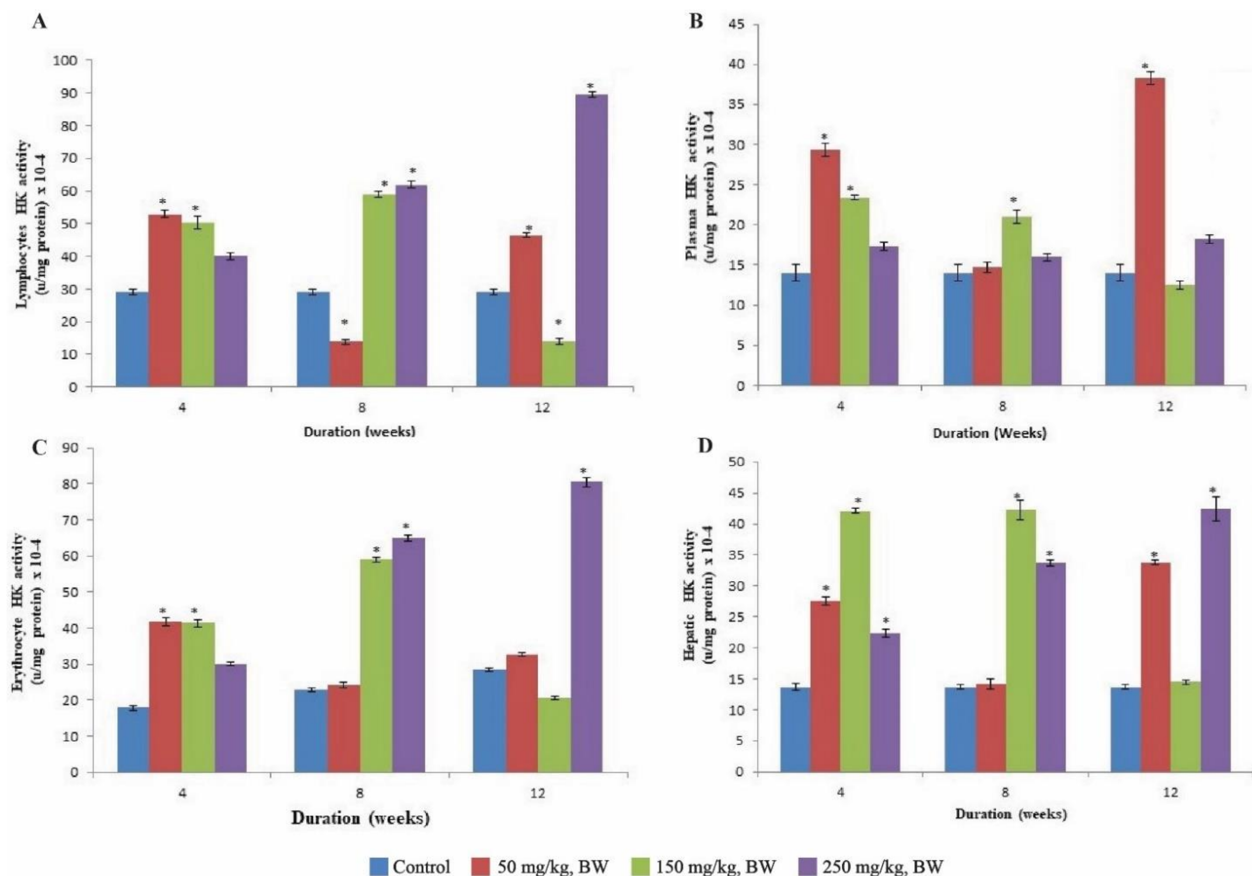
TiO_2-NPs induced a hormetic response characterized by an elevation in plasma hexokinase activity, with increases of 160%, 107%, and 54% at 4 weeks. At 8 weeks, doses of 50, 150, and 250 mg/kg BW led to increases of 47%, 110%, and 59% in plasma hexokinase, respectively. However, at 12 weeks, the 50 mg/kg dose resulted in an 84% increase, while significant decreases ($p<0.05$) were observed at 150 mg/kg and 250 mg/kg BW (39% and 12%) relative to the control group.

In erythrocytes, a 4-week exposure to TiO_2-NPs led to substantial increases (132%, 130%, and 67%, respectively) in erythrocyte hexokinase activity at 50, 150, and 250 mg/kg BW compared to the control group. After 8 weeks, no effect was observed at the 50 mg/kg BW dose, but there was an up-regulation of erythrocyte hexokinase activity at 150 and 250 mg/kg BW (2-fold each). At 12 weeks, an increase in hexokinase activity significant was noted at both 50 mg/kg BW (15%) and 250 mg/kg BW (2-fold), while a significant decrease was observed at 150 mg/kg BW (27%) relative with control.

Hepatic hexokinase activity showed consistent up-regulation throughout the 4-week exposure period, with a notably significant four-fold increase observed at 150 mg/kg BW. However, the 50 mg/kg BW dosage elicited a sinusoidal response. After 8 weeks, a 24% decrease was noted at 50 mg/kg BW, while at 150 mg/kg and 250 mg/kg BW, there were increases of 123% and 78%, respectively, compared to the control group. At 12 weeks, exposure to TiO_2 NPs at 50 mg/kg and 250 mg/kg BW resulted in increases of hepatic hexokinase activity by 75% and 120%, respectively, in contrast to the 24% down-regulation observed with 150 mg/kg BW.

Figure 1

Hexokinase activity



*Notes: Hexokinase (HK) activity in the lymphocytes (A), plasma (B), erythrocytes (C) and liver (D) of the animals exposed to TiO_2-NPs. Each value represent mean ± SEM of six animals. *significantly different ($p<0.05$) with control.*

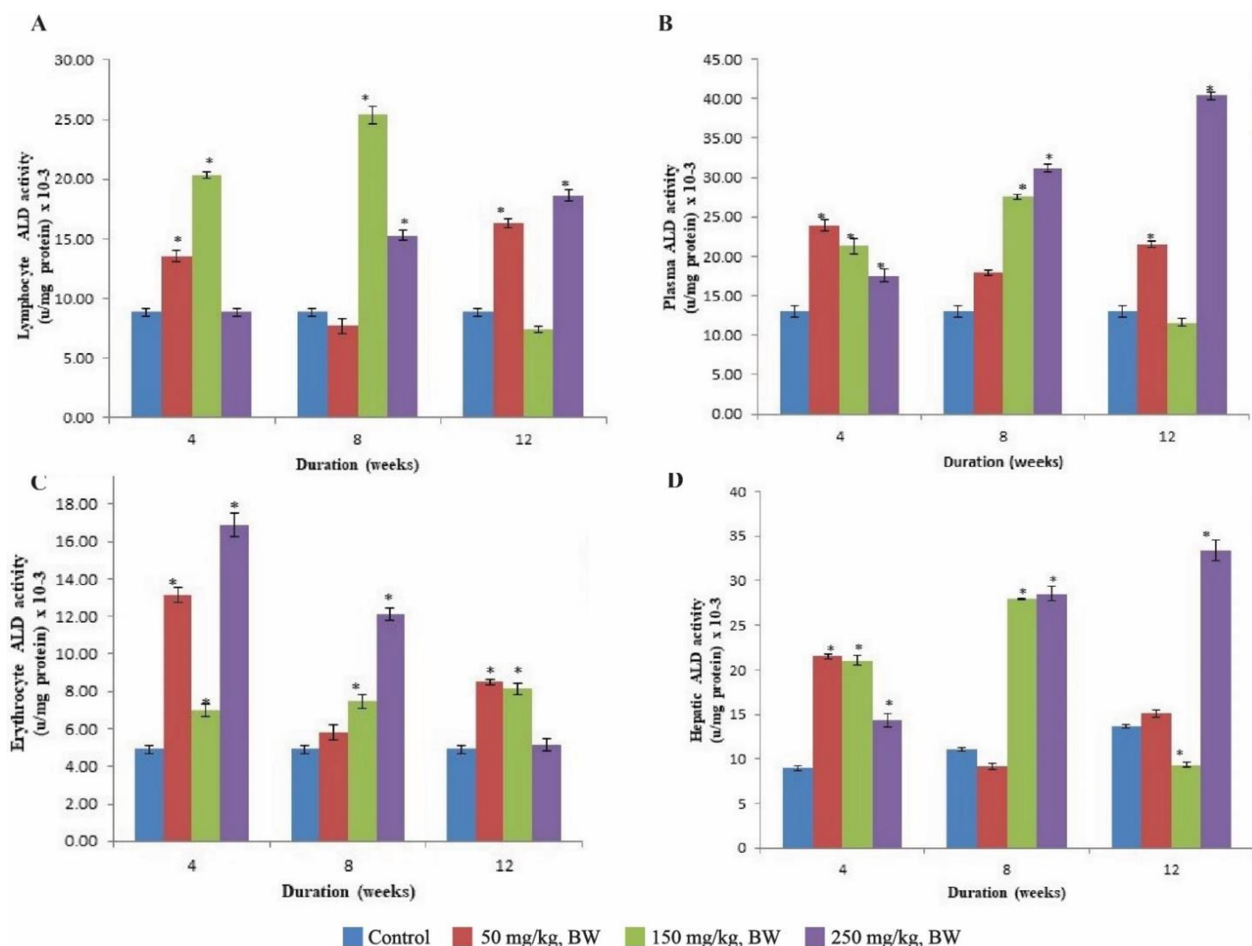
TiO_2-NPs perturbs aldolase activities in Wistar rats

As depicted in Figure 2, a significant increase ($p<0.05$) in lymphocyte aldolase activity was seen after 4 weeks of exposure to TiO_2-NPs, with the most substantial increase noted at 150 mg/kg BW (two-fold). There was no significant difference ($p<0.05$) in the activity of lymphocyte aldolase at 8 weeks with a 50 mg/kg BW dose. However, at this time point, a four-fold increase was seen with 150 mg/kg BW, and a two-fold increase with 250 mg/kg BW. After 12 weeks, increases of 40% and 60% were observed at 50 mg/kg and 250 mg/kg BW, respectively. However, a 36% decrease in aldolase activity was noted at 12 weeks with 150 mg/kg BW.

A hormetic response was evident in plasma aldolase levels due to TiO_2-NPs exposure at 4 weeks of exposure. While the 50 mg/kg BW dose increased plasma aldolase activity by 84%, the increase was 64% and 35% with the 150 mg/kg and 250 mg/kg BW doses of TiO_2-NPs. Additionally, during the 8-week exposure to TiO_2-NPs, a dose-dependent increase of 26%, 93%, and 119% was observed, respectively. At 12 weeks, an up-regulation of plasma aldolase was observed at 50 mg/kg BW (70%) and 250 mg/kg BW (100%), while a significant decrease ($p<0.05$) occurred with the 150 mg/kg BW.

Figure 2

Aldolase activity



*Note: Aldolase (ALD) activity in the lymphocytes (A), plasma (B), erythrocytes (C) and liver (D) of the Wistar rats exposed to TiO_2-NPs. The values are expressed as Mean ± SEM of rats (n = 6) in each group. *significantly different ($p<0.05$) with control.*

Exposure to all three doses of TiO_2-NPs for 4 and 8 weeks resulted in a significant increase in erythrocyte aldolase activity ($p<0.05$), although these increases did not exhibit a dose-dependent trend. Notably, at 8 weeks, a significant increase was also observed at both 150 mg/kg and 250 mg/kg BW (52% and 146%, respectively). However, during the 12-week exposure period, a dose-dependent decrease was observed (14%, 18%, and 48%, respectively).

A duration-dependent up-regulated aldolase activity of the liver was elicited by the 250 mg/kg BW dose of TiO_2-NPs. After 4 weeks of exposure, all doses led to up-regulated hepatic aldolase activities, with increases of 139%, 134%, and 59% compared to the control, respectively. An exception occurred at 8 weeks, where a 17%

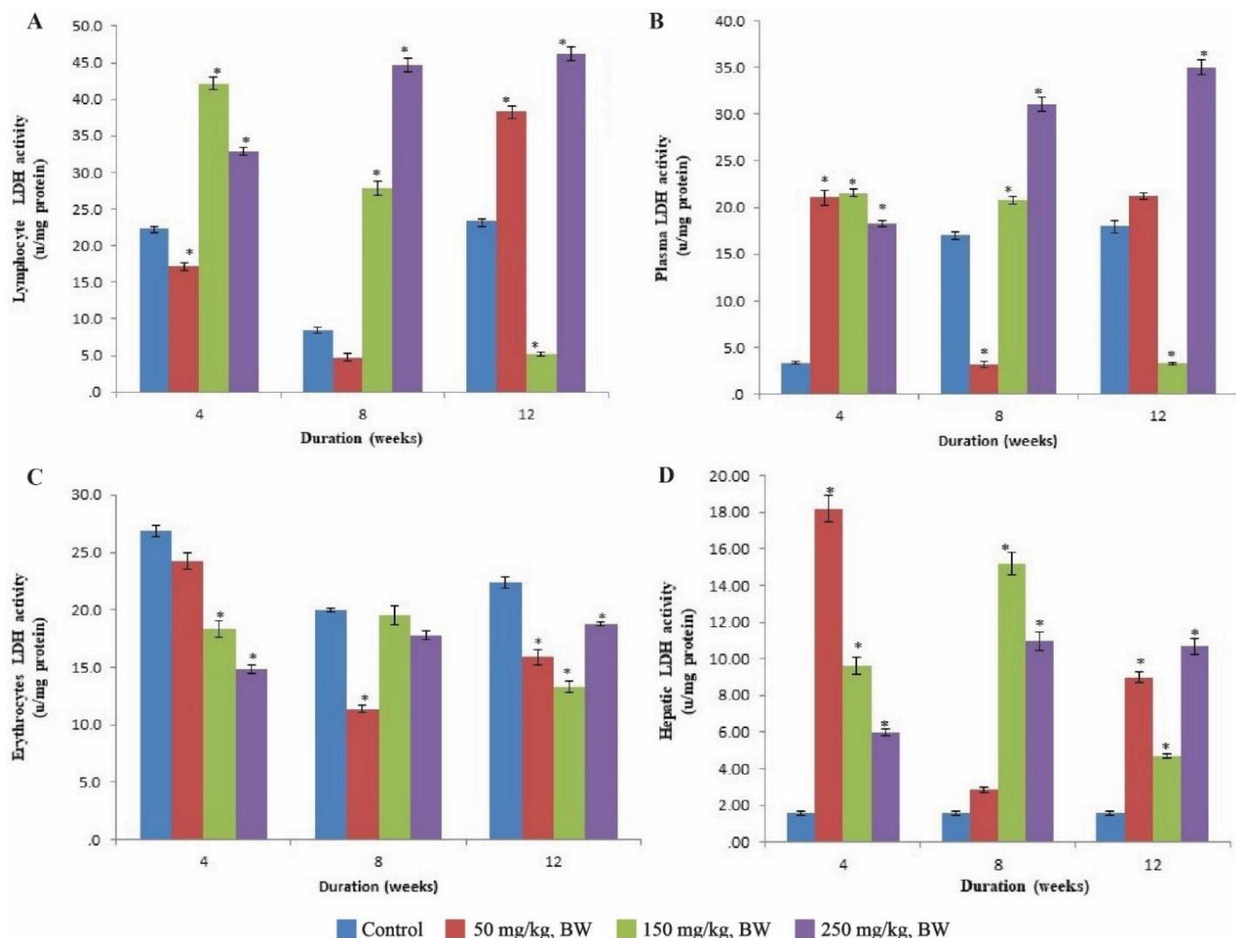
down-regulation of liver aldolase was noted for the 50 mg/kg BW dose, while increases of 152% and 158% were observed with the 150 mg/kg and 250 mg/kg BW doses, respectively. Finally, at 12 weeks, liver aldolase activity significantly decreased by 31% at 150 mg/kg BW, in contrast to the later increase of 144% observed at 250 mg/kg BW when compared to the control.

TiO2-NPs alters Lactate Dehydrogenase activities in Wistar rats

Figure 3 illustrate lymphocytes, plasma, erythrocytes, and liver lactate dehydrogenase activity in animals exposed to TiO_2-NPs. A duration-dependent increase in lymphocyte lactate dehydrogenase was induced by the 250 mg/kg BW dose of TiO_2-NPs. The increase was 47% at 4 weeks, 424% at 8 weeks, and 98% at 12 weeks, respectively. Although the 150 mg/kg BW of TiO_2-NPs also triggered a duration-dependent increase in lymphocyte lactate dehydrogenase, up to 8 weeks (89% and 224%, respectively), the 12-week exposure to 150 mg/kg BW of TiO_2-NPs resulted in a 78% decrease in enzyme activity. In contrast to the other two doses, the activity of lymphocyte LDH decreased by 23% and 45% at 4 and 8 weeks, respectively, when exposed to 50 mg/kg BW of TiO_2-NPs. However, a 63% increase in lymphocyte lactate dehydrogenase was observed by the 12th week of 50 mg/kg BW TiO_2-NPs.

Figure 3

Lactate dehydrogenase activity



*Note: Lactate dehydrogenase (LDH) activity in the lymphocytes (A), plasma (B), erythrocytes (C) and liver (D) of rats exposed to TiO2-NPs. The values are expressed as Mean ± SEM of rats (n = 6) in each group. *significantly different (p<0.05) with control.*

A duration-dependent decrease in plasma lactate dehydrogenase activity was noted with 150 and 250 mg/kg BW doses, except at 12 weeks with 150 mg/kg BW of TiO_2-NPs, where the enzyme activity was 18% of the control. The 50 mg/kg BW dose, on the other hand, induced a sinusoidal response from lactate dehydrogenase activity throughout the exposure period. Erythrocyte lactate dehydrogenase activity in animals exposed to TiO_2-NPs consistently decreased over the 12-week exposure period (9%, 31%, and 44%, respectively). While the decrease displayed a dose-dependent pattern at 4 weeks, the decreases observed at 8 and 12 weeks were not dose-dependent.

As illustrated in the figure, a 4-week exposure to TiO_2-NPs resulted in a dose-dependent increase (10-fold, 5-fold, and 3-fold, respectively) in hepatic lactate dehydrogenase activity. In contrast, an 8-week exposure to 50 mg/kg BW down-regulated liver lactate dehydrogenase by 57%. Moreover, at 12 weeks, exposure to 150 mg/kg BW led to a significant down-regulation (44%), while an increase of 26% was observed with 250 mg/kg BW compared to the control.

TiO_2-NPs perturbs NADase activities in Wistar rats

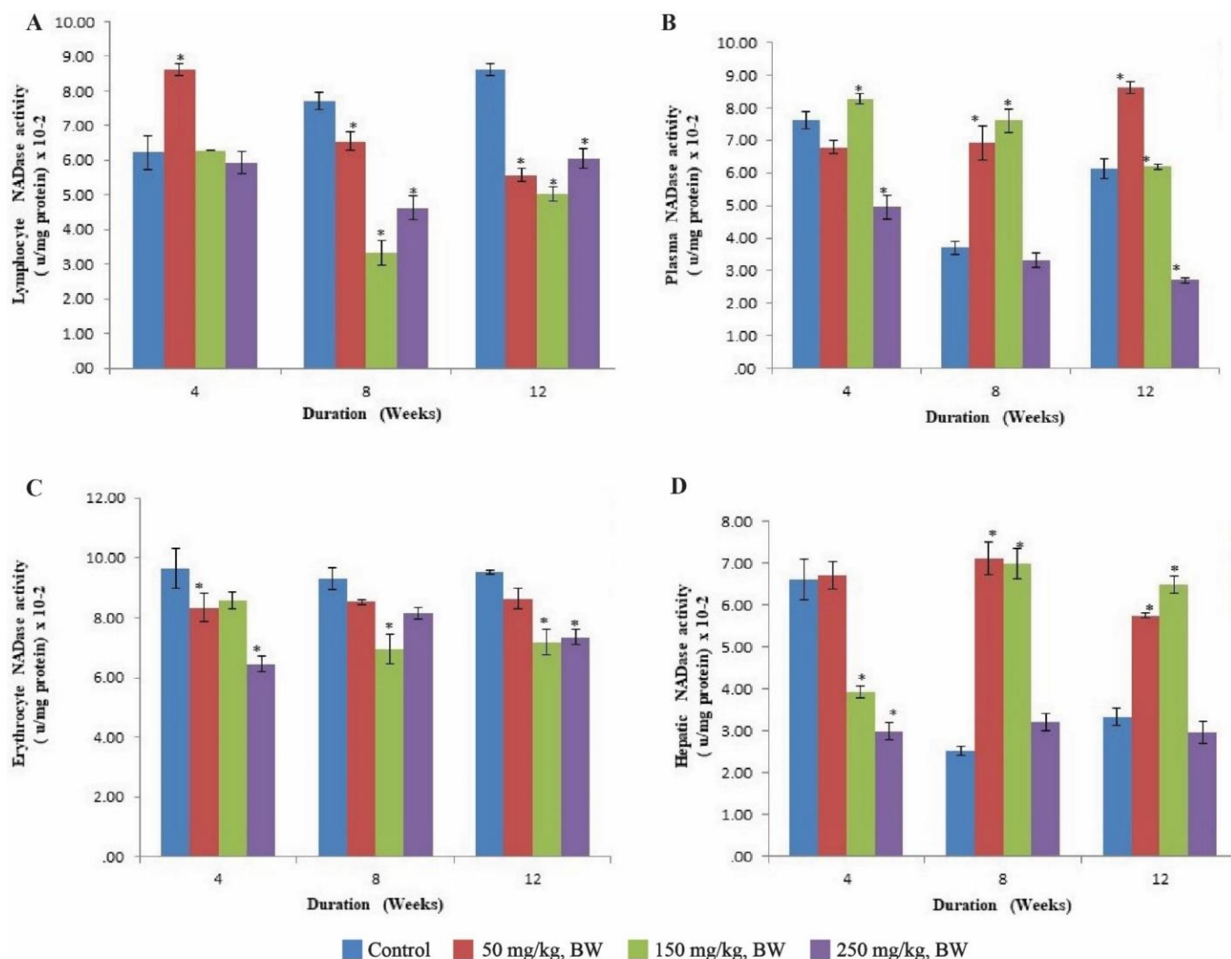
Figure 4 represent lymphocyte, plasma, erythrocytes, and hepatic NADase activity in the exposed animals. At 4 weeks, the 50 mg/kg BW dose caused an increase in lymphocyte NADase activity compared to the control. However, exposure to 150 and 250 mg/kg BW for 8 weeks resulted in a down-regulation of 56% and 40% in NADase activity. At 12 weeks, all the doses produced a down-regulation in lymphocyte NADase activity compared to the control.

At 4 weeks, 250 mg/kg BW reduced NADase activity by 35%. During 8 weeks, exposure to 50 and 150 mg/kg BW of TiO_2-NPs led to increases of 86% and 105%, respectively, in plasma NADase activity compared to the control. At 12 weeks, 50 mg/kg BW significantly increased plasma NADase activity by 40%, while 250 mg/kg BW inhibited the activity of the enzyme by 55% compared to the control.

A down-regulated activity was observed at all the doses of TiO_2-NPs during the exposure period, with the decrease being more pronounced with the 150 mg/kg BW dose of TiO_2 NPs. At 4 weeks, 150 and 250 mg/kg BW significantly decreased hepatic NADase activity by 40% and 54%, respectively. After 8 weeks, hepatic NADase activity was significantly increased upon exposure to TiO_2-NPs (181%, 176%, and 27%, respectively). Similarly, the activity of hepatic NADase was up-regulated (73% and 95%) after 12 weeks of exposure to 50 and 150 mg/kg BW of TiO_2-NPs when compared to the control, while it decreased upon exposure to 250 mg/kg BW of TiO_2-NPs.

Figure 4

NADase activity



*Note: Lactate dehydrogenase (LDH) activity in the lymphocytes, plasma, erythrocytes and liver of rats exposed to TiO_2 NPs. The values are expressed as Mean ± SEM of rats (n = 6) in each group. *significantly different ($p<0.05$).*

DISCUSSION

The findings of this study show that exposure to TiO_2-NPs causes disruptions in energy metabolism by modulating glycolytic enzymes in the cytosol. These perturbations are characterized by an increase in the activity of several glycolytic enzymes in lymphocytes, plasma, and the liver. Hexokinase, the initial enzyme in the glycolytic pathway responsible for facilitating glucose entry into cells through phosphorylation to produce glucose-6-phosphate, represents a potential target for toxicants able of perturbating cellular energy transduction (Kavanagh Williamson et al., 2018). In this study, the activity of hexokinase in lymphocytes increased following exposure to TiO_2-NPs. This observation suggests the potential immune-stimulatory effects of TiO_2-NPs. Lappas (2015) proposed that exposure to TiO_2-NPs leads to multiple immunomodulatory potentials, orchestrated by alterations in immune cell numbers, viability, and function, and the accumulation of nanoparticles in peripheral lymphoid organs. The increased activity of these enzymes implies a higher energy demand for immune cells, which may be necessary for their differentiation into plasma cells and antibody production, ultimately aiding in defending the body against potential threats associated with TiO_2-NPs exposure (Olajide et al., 2023). The notably high hexokinase activity in immune cells suggests that TiO_2-NPs could potentially act as immunogens. Exposure to immunogens has been shown to enhance glucose uptake, consequently increasing hexokinase activity in the process (Jo et al., 2015). This observation aligns with the increased plasma, erythrocyte, and hepatic hexokinase activities observed at week 4 of exposure to TiO_2-NPs.

Aldolase, an enzyme that facilitates the reversible break of phosphorylated fructose 1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehyde-3-phosphate (Perovich et al., 2021). It is routinely assessed to evaluate various myopathies. Our study revealed significant increases in aldolase activity in lymphocytes, plasma, liver, and erythrocytes when compared to the control across all doses at week 4. The up-regulation of aldolase activity after exposure to TiO_2-NPs is not surprising as Mamczur et al. (2013) demonstrated that elevated aldolase activity is associated with highly proliferating cells. This suggests that the increased aldolase activity in these compartments could be a subtle mechanism employed by the system to stabilize cellular functions. It is noteworthy that the toxic effects of 150mg/kg BW TiO_2-NPs on hepatic cell at the 12th weeks with the observed down-regulation of aldolase activity may be as a result of the cell not being able to fully cope with the increased energy demand impacted by TiO_2-NPs. Also, aldolase down-regulation will limit the flux of metabolite along the glycolytic pathway and glyceraldehyde-3- phosphodehydrogenase could be inhibited by TiO_2 being an analog of phosphorus because of its oxidation state (+4). The phospho-enzyme (GHAPDH) could be inhibited by TiO_2-NPs thereby making NADH unavailable for the enzyme lactate dehydrogenase (LDH) reaction to proceed.

The enzyme lactate dehydrogenase (LDH), which is found in a number of tissues including the blood, pancreas, liver, heart, kidney, skeletal muscle, and lymph tissue, is necessary for the reversible conversion of pyruvate to lactate (Miranda et al., 2018). Inadequate blood flow, cerebrovascular accidents, hemolytic anemia, liver illness, and tissue injury are among the diseases that are associated with high LDH activity in tissues (Miranda et al., 2018). For cells that lack mitochondria, LDH activity represents an index for determining not just the membrane permeability but also as a sensor for energy homeostasis (Miranda et al.,2018). Red blood cells rely exclusively on glycolysis for ATP production, which is crucial for their metabolic processes and maintenance of membrane integrity, as well as providing energy for ATPases (Ghashghaeinia et al., 2019). Although the observed decrease in LDH activity in erythrocytes might suggest damage to these cells, Kose (2020) reported the binding of TiO_2-NPs to LDH, which may reduce its activity. Whether this reduction in activity has functional consequences in erythrocytes remains to be fully understood as decreased activity of lactate dehydrogenase in the erythrocytes is not expected but the erythrocyte is susceptible to varying degree of toxicity because of its many roles in transporting materials. This transport function makes the RBC to become potential target for toxic substances. However, marked increments were observed in the activity of LDH in the lymphocytes, plasma and hepatic cell at all doses in the present study. While it has been shown that high lactate levels inside the tumor bed can "stun" T cell responses, it is also hypothesized that elevated LDH may represent fast cell turnover of aggressive disease as tumour cells favour glycolysis over oxidative phosphorylation even in the presence of oxygen (Romero-Garcia et al., 2016)

Nicotinamide and ADP-ribose are generated when NAD+ is hydrolyzed by NAD^+ glycohydrolase (NADase). The action of this membrane-bound enzyme is restricted to the membrane's outer surface (Poltronieri et al., 2021). There is little information in the literature about how TiO_2-NPs affect NADase activity. This investigation looked at how TiO_2-NPs affected the activity of NAD^+ glycohydrolase in various organs. Relative to control, the NADase activity of erythrocytes and lymphocytes was considerably suppressed in this study. The observed inhibition of NADase in these tissues may signify a lower rate of breakdown given that cytosolic content of NAD^+ is normally minimal and that the rate its concentration and breakdown are carefully regulated due to its involvement as an electron acceptor in several oxidative pathways (Kehinde et al., 2023). However, the down-regulated activity of NADase seems to potentiate the glycolytic pathway, as glycolysis was stimulated by TiO_2-NPs (Cantóet al., 2015).

CONCLUSION

Our results reveal elevated hexokinase and aldolase activities in animals exposed to TiO_2-NPs, indicating an increased demand for energy, often met by an upregulation of glycolysis. Our findings indicate increased hepatic hexokinase and aldolase activities in animals exposed to TiO_2-NPs, suggesting an increased demand for energy, usually met by increasing the rate of glycolysis. This heightened hepatic glycolysis might indicate hepatic regeneration during TiO_2-NPs exposure. It could represent an adaptive response by the liver to maintain homeostasis and prevent hepatotoxicity induced by TiO_2-NPs during the time-course under investigation. Further investigations are needed to unveil the functional implications of this shift in energy metabolism in various organs when exposed to TiO_2-NPs, considering other pathways of energy metabolism.

AUTHOR CONTRIBUTIONS

Ademuyiwa Oladipo conceived the work; Kehinde Samuel Abiodun carried out literature search and all experimental work, performed statistical analysis and data interpretation and wrote the draft of the manuscript. Ademuyiwa Oladipo, Ugbaja Regina Ngozi, Dare Enock Olugbenga and Adelesi Oluwatobi Gabriel contributed to design, analysis and interpretation of data and critical review of the manuscript. Ademuyiwa Oladipo, Ugbaja Regina Ngozi and Dare Enock Olugbenga supervised the work and contributed intellectual input in the discussion and overall presentation of the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL

The Departmental Ethical Review Committee of the College of Biosciences of Federal University of Agriculture, Abeokuta approved the study (FUNAAB/CBS/BCH/141014).

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests in this work.

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