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# Ameliorative Ability of *Cyperus esculentus* (Tiger Nut) and *Phoenix dactylifera* (Date Palm) Fruits on Blood Haematology and Film of Wistar Rats in Titanium Dioxide Nanoparticles Ingestion Episode

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**ABSTRACT**: The present study investigated the ameliorative ability of *Cyperus esculentus* and *Phoenix dactylifera* fruits on blood haematology and film of wistar rats in titanium dioxide nanoparticles ingestion episode. 25 rats randomly distributed into five (5) groups were orally administered with varying combinations of titanium dioxide and tiger nut and date palm fruits extracts for 60 days, after which blood was collected for hematological and blood film analysis following standard methods. Hematological analysis showed that titanium dioxide nanoparticles caused a decrease in white blood cell (6.52%), granulocyte (56.06%), hemoglobin (3.10%) and hematocrit (15.47%) with an increase in lymphocyte (0.52%), monocyte (48.51%), red blood cell count (7.53%), platelet count (24.21%) and plateletocrit (37.93%) of wistar rats when compared with the control. Leishman-stained blood film revealed the presence of mild to moderate dimorphic anaemia, polychromatic morphology with some macrocytic cells and aggregated thrombocytes in the blood film of rats administered $TiO_2NP$. There was no significant ameliorative impact of *C. esculentus* and *Phoenix dactylifera* extract against $TiO_2NP$ toxicity. This may be due to the small particle size and reactive nature of titanium dioxide nanoparticles. The findings of this study indicate that titanium dioxide nanoparticles negatively impacted blood hematological parameters in male wistar rats. Titanium dioxide nanoparticles should be handled and treated carefully to avoid potential health hazard in animals.

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Following recent technological development, titanium dioxide ($TiO_2$) nano-particles have been exclusively developed as well as widely applied in a broad variety of products including plastics, paper, cosmetics, ink and medicine for diagnosis. Some research has found $TiO_2$ in a range of consumer and industrial products, including paints, coatings, adhesives, Paper rubber, toothpaste, soap, food colorants, pharmaceuticals, sunscreen and catalysts (Lu *et al.*, 2018, Medina *et al.*, 2007; Jiang *et al.*, 2008; Pandey *et al.*, 2017, Shi *et al.*, 2013) with humans estimated to use approximately 300mg of titanium per day with commercial food items (Dunford *et al.*, 1997). Moreover, $TiO_2$ has been proven to be efficient in killing antibiotic resistant bacteria by destroying bacterial spores (Brunet *et al*, 2009). The advantageous combination of its biological and physico-chemical properties resulted to titanium being extensively used for a broad range of implanted medical devices, for example, dental implants, joint replacements (Chen *et. al.*, 2009). It is well documented that nanoparticles are able to cross the blood–testes and blood–brain barriers (De Jong *et al.*,

2005; Lankveld *et al*., 2010). In the past few decades, the effect of titanium dioxide nanoparticles ($TiO_2$NPs) on human health has given rise to serious concern. This makes it an obvious reason for this nano-sized material to be study, most especially their biological effect as they can pass through cell membrane easily and even pass through blood-brain barrier and blood-testes barrier (McAuliffe *et al*. 2007), which can affect other vital organs such as the kidney, heart, liver through blood circulation. These particles are known to exhibit toxic effects due to their particle size and high surface area (Carlson *et al*., 2008).
Natural antioxidants have recently attracted considerable attention for preventing oxidative stress-related diseases including cancers, cardiovascular diseases and degenerative diseases (Ogura *et al*., 2008). Some researchers found *Cyperus esculentus* (CE) and *Phoenix dactylifera* (PD) *to* be a fertility booster, to stimulate sexual motivation and improve sexual performance in rats (Jeong *et al*., 2009) and it has also been presented to help attenuate sperm toxicity thereby improving sperm count and to activates blood circulation (Trinidad *et al*., 2010; Badejo *et al*., 2014; Ekaluo *et al*., 2015). In Nigeria, it is common folklore believe that the consumption of fruit extract of CE and PD improves sperm quality in men. The broad applications of titanium dioxide nano-particles have raised serious concerns about their biological effects as humans are potentially exposed to nano-particles (NPs). As a result, health risk assessments of nanoparticles have become mandatory in order to better protect humans. Potential hematological changes are relevant for this risk evaluation. This study evaluated the ameliorative ability of *Cyperus esculentus* and *Phoenix dactylifera* fruits on blood haematology and film of wistar rats in the event of titanium dioxide nanoparticles ingestion.

## MATERIAL AND METHODS

*Test substance and preparation of $TIO_2$-NPs stock solution:* Titanium dioxide nanopowder [($TiO_2$-NPs, anatase, CAS number: 1317-70-0, product code - 637254), Purity: 99.7%, Average Particle Size: <25 nm, Specific Surface Area: 45 $m^2$/g, Color: white, Morphology: powder and relative density: 3.9g/mL] was obtained commercially from Sigma Aldrich Co. Germany.

This nanoparticles was chosen because of its utilization in previous studies (Bakare *et al*., 2016), and its physico-chemical characterization has been previously reported by Shukla *et al*. (2011). The $TiO_2$-NPs were suspended in distilled water at a concentration of 200 mg/kg body weight. The mixture was vortex for five minutes to disperse the particles before it is administered.

*Collection, identification and laboratory analysis of fruits:* Fresh fruits of *Cyperus esculentus* (CE) and *Phoenix dactylifera* (PD) were purchased from the local market in Effurun, Delta state; the taxonomic identity of the fruit was confirmed at the Department of Environmental Management and Toxicology, College of Science, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria. Phytochemical analysis (such as alkaloids, tannin, saponin, cyanogenic glycoside and flavonoid) of the crude fruit extracts was determined according to the standard procedures to identify the constituents as described by (Sofowora, 1982; Trease and Evans, 1989; Kokate *et al*., 2008; Harborne, 1988).

*Preparation of fruit extracts:* In this study, two fruits were used. These includes *Cyperus esculentus* (CE) and *Phoenix dactylifera* (PD). The fresh fruit were shade-dried and crushed (the date palm seeds were removed) into fine powder. The crude aqueous extract was prepared by dissolving 20g each of the pulverized CE and PD in 50ml and 250ml of distilled water respectively for 48hrs; The mixture was decanted and filtered using sterile whatman paper No 1. The filtrate was evaporated to dryness using a freeze dryer and reconstituted in distilled water to appropriate concentrations.

*Experimental setup:* Thirty (30) male wistar rats (6-7 weeks old) weighing within the range of 100g to 150g were obtained from the Anatomy Department, University of Benin, Nigeria. The rats were distributed randomly into five groups; group A to E with each group containing five (5) male rats and were allowed to acclimatized for 2 weeks until they were 8-9 weeks and their weights taken. The animals were housed in wooden cages with wire mess covers. The animals were fed with standard rodent chow (Bendel Livestock Feeds Limited, Ewu, Edo state, Nigeria) and given distilled water *ad labium*. After acclimatization, the Group A was designated as control (CC) while group B – E was given 200mg/kg b/w $TiO_2$NP (TT), 200mg/kg b/w $TiO_2$NP + 200mg/kg b/w CE (TA1), 200mg/kg b/w $TiO_2$NP + 400mg/kg b/w PD (TA2) and 200mg/kg b/w $TiO_2$NP + Combination (consisting 200mg/kg b/w CE, + 400mg/kg b/w PD) (TA3) respectively (once every 48 hour) for 60 consecutive days.

The rats were maintained in laboratory conditions during exposure; and had access to drinking water and standard rodent chow (Bendel Livestock Feeds, Ewu, Edo state, Nigeria®) *ad libitum*. After exposure, survivors were fasted overnight and sacrificed under slight Anesthesia; then blood samples were collected for analysis.

*Collection and preparation of samples:* Blood was collected from the inferior vena cava of the rats with plain 5ml sterilized syringe into a vial containing 0.5m EDTA for haematological analysis under a light anaesthesia. The blood sample was placed gently in a cooler containing ice and transferred to the laboratory for analysis.

*Laboratory Analysis:* Haematological analysis was carried out using Sysmx KX-21N automated machine (Sysmx corporation kobe, Japan) following the manufacturer's instructions. Briefly the sample was mixed and placed in contact with the sample probe for aspiration, when the buzzer sounds twice 'beep, beep' and when the LCD screen displays ANALYZING, sample was removed. Following this, the unit executed automatic analysis, and the result was display on the LCD screen and printed out. Haematological parameters including white blood cell count (WBC), percent of lymphocytes (LYM%), percent of monocytes (MID%), percent of granulocytes (GRAN%), erythrocytes (RBC), haemoglobin (HGB), (HCT), platelet count (PLT), mean corpuscular volume (MCV), (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), as well as total platelet crit (PCT) were analyzed.

A drop of blood previously store in an EDTA bottle was placed on a slide. The blood was spread using the cover slip and left to dry at room temperature. The dried film was stained using Leishman stain and left for 30 minutes. The film was rinsed with water and dehydrated using ascending grades of alcohol (starting from 70%, 90%, 96% and absolute); and cleared in xylene for 5 minute. The section was mounted using shandom's mount (DistreneDibutylPhathalate xylene), covered with a cover slip and allowed to dry. The slides were examined using Leica CME light microscope (Model – 1349522X).

*Statistical Analysis:* All statistical analyses were conducted with Statistical Package for Social Scientists (SPSS) and Microsoft Excel computer software. Data are presented as mean ± SE (n=5/sex). One-way ANOVA was explored to determine the differences among various groups.

## RESULTS AND DISCUSSION

The results of the phytochemical screening of the crude aqueous fruit extract of *Cyperus esculentus* and *Phoenix dactylifera* showed the existence of various secondary metabolites such as alkaloids, tannin, saponin, cardiac glycoside and flavonoid in varying concentrations (Table 1**).** However, flavonoids was absent in CE while abundant in PD.

**Table 1:** Phytochemical composition of *Cyperus esculentus* and *Phoenix dactylifera*.

| | Parameter | *Cyperus esculentus* | *Phoenix dactylifera* |
|---|---|---|---|
| 1. | Alkaloids | +++ | ++ |
| 2. | Tannin | ++ | ++ |
| 3. | Saponin | + | + |
| 4. | Cardiac glycoside | + | + |
| 5. | Flavonoids | - | +++ |

*NB: - indicates absent; + trace ++ indicates moderately high; +++ present in abundance*

The results of the haematological studies is as shown in table 2. There were no significant difference in white blood cells (WBC), and lymphocytes (LYM) in group of rats administered $TiO_2$NP (TT group) as compared to control group. However, a significant increase in monocytes (MID), Plateletcrite (PCT), platelet (PLT), red cell distribution width (RDW-CV), and mean corpuscular hemoglobin concentration (MCHC) was noticed in TT group when compared with control and the reverse was for HCT and MCV. Additionally, no significant difference in red blood cells (RBC), hemoglobin (HGB) mean platelet volume (MPV), red cell distribution width (RDW-SD), and mean corpuscular hemoglobin (MCH) was seen across all groups (CC, TT, TA1, TA2, TA3). Moreso a significant decrease in LYM, MID, MCHC, PLT was seen TA1, TA3 group as against TT group, with the reverse in the case for GRAN, HCT and mean corpuscular volume (MCV). Also no significant difference was observed in MCHC, and RDW-CV in groups of rats administered with the different abatements co-administered with $TiO_2$NP (TA1, TA2, TA3) as compared with TT group. There were no significant difference in MCV, GRAN, MCHC, and RDW-CV in the different abatements co-administered with $TiO_2$NPs. Leishmian stained blood film of the control group and the group of rat exposed to $TiO_2$NP, TA1, TA2 and TA3 is shown from plates 1a to 1e respectively. The control group showed a normocytic, normochromic red blood cell structure which signifies a normal and erythropoeitic function. The thrombocytes showed normal formation and count as seen in (Plate 1a). Plate 1b showed a Dimorphic, Polychromatic red cells. Plate 1c showed spherocytes, polymorphic red cells, aggregated thrombocytes. Plate 1d showed Macrocytic, Dimchromic cells, and plate 1e, a normocytic, normochromic cells, thrombocytosis.

**Table 2:** Changes in blood indices associated with the oral administration of crude extracts of *Cyperus esculentus* and *Phoenix dactylifera* and $TiO_2NP$ in male wistar rats

| | CC | TT | TA1 | TA2 | TA3 |
|---|---|---|---|---|---|
| WBC ($\times 10^9$ cells/L) | 4.60±0.10[a] | 4.30±0.20[a] | 4.55±1.05[a] | 3.05±0.15[b] | 4.05±2.75[a] |
| LYM (%) | 86.70±1.55[a] | 87.15±3.05[a] | 85.05±4.95[a] | 87.25±5.35[a] | 78.55±3.85[b] |
| MID (%) | 6.70±0.55[a] | 9.95±2.95[b] | 7.50±2.70[a] | 6.45±1.55[a] | 13.55±1.65[b] |
| GRAN (%) | 6.60±2.10[a] | 2.90±0.10[b] | 7.45±2.25[a] | 6.30±3.80[a] | 7.90±2.20[a] |
| RBC ($\times 10^9$ cells/L) | 6.11±0.51 | 6.57±0.11 | 6.56±0.11 | 6.17±1.04 | 6.66±0.06 |
| HGB (g/dL) | 14.50±1.00 | 14.05±0.25 | 15.30±0.30 | 14.15±1.95 | 15.60±0.00 |
| HCT (%) | 40.40±0.85[a] | 34.15±0.35[b] | 39.40±3.80[a] | 36.90±1.70[b] | 40.65±2.55[a] |
| MCV ($\mu m^3$) | 66.20±0.10[a] | 52.05±2.05[b] | 60.25±0.40[a] | 61.15±0.10[a] | 61.25±0.20[a] |
| MCH (ρg) | 23.70±1.55 | 21.35±2.40 | 23.30±1.30 | 23.00±1.55 | 23.40±3.05 |
| MCHC (g/dl) | 35.80±0.55[a] | 41.10±1.70[b] | 39.10±2.30[b] | 38.15±0.55[b] | 38.50±2.95[b] |
| RDW-CV (%) | 24.10±2.10[a] | 27.80±0.70[b] | 27.95±1.00[b] | 27.95±2.10[b] | 29.00±0.10[b] |
| RDW-SD ($\mu m^3$) | 13.40±0.51 | 13.80±0.66 | 14.35±0.06 | 14.20±0.10 | 14.75±0.11 |
| PLT ($\times 10^9$ cells/L) | 475.00±9.00[a] | 590.00±0.00[b] | 486.00±62.00[b] | 408.50±100.50[c] | 446.00±11.00[b] |
| PCT (%) | 0.29±0.03[a] | 0.40±0.02b | 0.33±0.03b | 0.28±0.08a | 0.30±0.01[a] |
| MPV ($\mu m^3$) | 6.30±0.85 | 6.80±3.45 | 6.85±1.55 | 6.90±1.00 | 6.75±0.35 |

*Values are means +SEM n = 5; Means with same superscript along the longitudinal axis are not significantly different.*

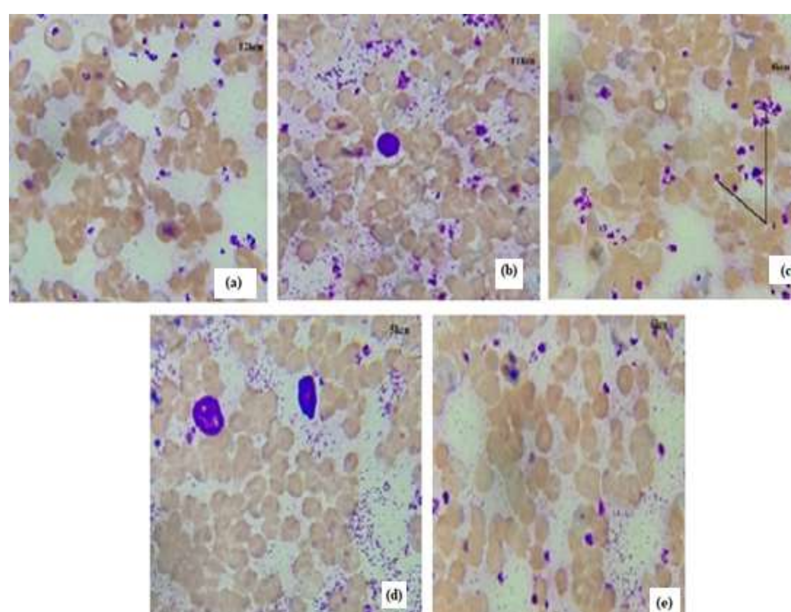

**Plate 1:** Blood film of (a) control male rat (b) rat exposed to $TiO_2NP$ **(c)** rat exposed to $TiO_2NP$ + C. *esculentus* (d) rat exposed to $TiO_2NP$ + *P. dactylifera* (e) rat exposed to $TiO_2NP$ + C. *esculentus* + *P. dactylifera* (Leishmian stain X 100)

The knowledge of haematological studies is a vital tool, which can be applied as a sensitive index to observe the internal environment of animals and humans (Vasantharaja *et al*., 2015) and to detect the hematological toxicity of different chemicals. Haematological constituents reflect the physiological responsiveness of the animal to its internal and external environments which include feed and feeding (Esonu *et al*., 2001). For the assessment of DNA integrity, blood cells are consistently used as a mirror of damage in other body cells, especially in non-mammalian vertebrates (Barata *et al*., 2010). Together with determining their pathogenicity, the detection of infectious agents in blood samples enables testing for ecological and evolutionary hypothesis, as well as increasing our understanding of their disease dynamics (Arnal *et al*., 2014). The white blood cell count (WBC) is usually carried out to provide information on the proportion of the different white cells present in circulating blood (Cheesbrough, 2002). One major function of granulocytes (neutrophils) is the uptake and killing of bacterial pathogens. They are the first responders to inflammation and cell damage by producing free radicals to remove damaged cells. Monocytes help the immune system fight infection. These white blood cells have the ability to turn into dendritic cells and macrophages when the immune system detects a foreign substance (Ugochukwu, 2003). The findings of this study indicated no significant difference in WBC, LYM, in groups of rats treated with $TiO_2NPs$ when compared with control group, however a significant increase was observed for MID with the reverse for GRAN. The low granulocytes level

observed in this study might be as a result of impaired immune response and vulnerability to infections. Increased monocyte count might be a suggestive of a chronic infection, an autoimmune or a blood disorder (Shugaba *et al*., 2012). These results contradict a research conducted by Bu *et al*. (2010) where $TiO_2NPs$ was shown to induced lymphocytsis and granulocytosis. According to them, the increased white blood count, lymphocyte and eosinophils were as a result of the titanium dioxide administration. $TiO_2NPs$ has been predicted to cause reduction of cellular antioxidants, inhibition and stimulation of oxidation stress in cell activity (Nemmar *et al*., 2008). The primary function of the red blood cells (erythrocytes) is to carry oxygen bound to hemoglobin from the lungs to the tissue. The hemoglobin in erythrocytes is an excellent acid-base buffer most responsible for the buffer capacity of whole blood (Ersley and Gabuzda, 1985). The present study cause a significant decrease in HCT and MCV with an increase in RDW-CV and MCHC in $TiO_2NPs$ groups as against control group. Moreover, no significant difference in red blood cells (RBC), hemoglobin (HGB) mean platelet volume (MPV), red cell distribution width (RDW-SD), and mean corpuscular hemoglobin (MCH) was seen across all groups (CC, TT, TA1, TA2, TA3). This is also in accordance with blood film of rats administered $TiO_2NP$ (Plate 2). The red cells exhibit a dimorphic and polychromatic morphology, with some macrocytic cells. This synchronizes with the physiological response as the mean concentration of HCT (34.15±0.35) seems to drop when compared with the control group. The extract seems to impact negatively on erythropoiesis and the morphology of the red cells which may impact the heamoglobin capacity, resulting in dimorphic anaemia. This might be due to iron deficiency and nutritional macrocytic anemia. Decrease in hematocrit may be due to suppressed bone marrow hematopoietic system, causing iron deficiency in synthesis of haem protein of hemoglobin (Klauder and Petering, 1977; guyton and Hall, 1996). The low hematocrit would also indicate anemia or oligoheamia (Wepener *et al*., 1992). This disorder would be as a result of low hemoglobin content, insufficient red blood cell and abnormal hemoglobin. This contradicts the findings of (Heba *et al*., 2021) where marked reductions in red blood cell counts (RBC), HGB, HCT, and WBCs were noticed in groups of African catfish treated with silver nanoparticles (AgNPs). However, the increased HCT aligns with this study. Similar findings have also been reported by Hounkpatin *et al*. (2013) which revealed a significant decrease in red blood cells (RBC), haemoglobin concentration (HGB) in wistar rats as a result of toxicity of cadmium, and mercury. Moresol, a significant reduction in mean platelet volume (MPV), and MCV was observed in groups of male wistar rats administered AgNPs (Ji Hyun *et al*., 2018). Again, Nikolic *et al*. (2013) showed that Pb, Cd and Cu intoxication significantly decreased values of erythrocytes, hemoglobin and HCT in treated wistar rats.

Rats exposed to $TiO_2NP$ + C. *esculentus* slides (plate 3) showed Spherocytes, and polymorphic red blood cells with cells lacking central pale area or eccentric pale area. This typically can be seen as an indirect physical or chemical injury or in cases of hereditary autoimmune hemolytic anemia. This is in alignment with the increase in MCV and HCT in TA1 group as compared with $TiO_2NPs$ group. The increased MCV in $TiO_2NP$ treated rats suggest that $TiO_2NP$ may cause a kind of macrocytic anemia in rats and also can be due to DNA damage which causes an interruption in mitotic period (Vasantharaja *et al*., 2015; Duan *et al*., 2010). In (Plate 4) $TiO_2NP$ + P. *dactylifera*, there was an observable mild to moderate dimorphic anaemia. The red cells exhibited a dimorphic and polychromatic morphology, with some macrocytic cells. This result also debunks the ameliorative properties of P. *dactylifera* therapy on the red blood cell indices as it showed little or no significant difference when compared with the $TiO_2NP$ group and the control group respectively. Also, exposure to $TiO_2NP$ + C. *esculentus* + P. *dactylifera* (plate 5) showed normocytic and normochromic red cells with a mean HCT of 40.65±2.55, and mean red blood cell count of 6.66±0.06. The combine therapy of C. esculentus + P. *dactylifera* seems to have a more potent synergistic ameliorative property than singularly as seen in plates 3 and 4 and when compared with the control group (plate 1). The red cells seems to take their normal shape, with normal HCT. This shows that there is a significant positive effect of the extract used together as palliative for the management and treatment of $TiO_2NP$ toxicity exposure. Platelets are cytoplasmatic fragments of bone marrow megakaryocytes (Laki, 1972). They are dynamic blood particles whose primary function, along with the coagulation factors, is haemostasis, or the prevention of bleeding. Platelets interact with each other, and also with leukocyte (LYM) and endothelial cells, searching for sites of injury, where they become activated (Machlus *et al*., 2014). In addition to their important role in haemostasis and thrombosis, accumulating evidence demonstrates that platelets contribute to the inflammatory process, microbial host defense, wound healing, angiogenesis, and remodelling (Jain, 1975). This study revealed increase in PLT, and PCT in titanium dioxide nanoparticles treated rats resulting to thrombocytosis with a significant decrease in PLT in TA1, TA2, and TA3 groups. Thromobocytosis may be

caused by anemia due to iron deficiency or infection (Cleveland clinic, 2017) which can result into certain conditions such as stroke, a cloth in the blood vessel and heart attack (Albert, 2005). The thrombocytes appear aggregated with severe thrombocytosis with an average Platelet count of 590.00±0.00 as compared with the control group (Plate 2). This thrombocytosis may be a reactive thrombocytosis since it was induced by $TiO_2$NP therapy. This results is in consonance with previous works done (Duan et al., 2010) who proved that $TiO_2$NP caused an increase in platelet count. It is suggestive that therapy with $TiO_2$NP can induce toxic effect on the endothelial functions which may laggregatedead to segregation of Platelets and in turn may impair systemic functions (Donaldson *et al*., 2001 and Bihari *et al*., 2010). Moreso, the thrombocytes (plate 3) appear with mild to moderate thrombocytosis with a mean average Platelet count of 486.00±62.00, as compared with the control group. However, when compared with $TiO_2$NP group, there seem to be no significant ameliorative property of C. esculentus extract. This may be due to the irreversible chemical injury caused by $TiO_2$NP administered orally. Similarly an increase in platelet counts was reported in wistar rats administered with Rutile Fe-doped nanorod titanium dioxide, causing a severe damage of PLTs but improving the metabolic function of the bone marrow (Neemmar *et al*., 2008). This was in accordance with a study conducted by (Duan *et al*., 2010) who observed an increase in PLT and MPV. According to them, the increase may be due to the possible effect of $TiO_2$NPs on blood coagulation. Plate 5 showed a normal thrombocytes count of 446.00±11.00 indicating the protective role of C. *esculentus* + P. *dactylifera* again $TiO_2$NP toxic effects in male wistar rats. Different plant based food and herbs have been reported to be beneficial to humans due to their phytochemical substances and *Cyperus esculentus* and *Phoenix dactylifera* happens to be among. Phytochemicals help to protect us against many related food diseases. A study on the antioxidant activity of Tiger nut indicated that it could be utilized to "mop up" and scavenge free-radicals, generate essential metabolic body reactions (Ogunlade, 2015). The results of the phytochemical screening of the aqueous fruit extract of *Cyperus esculentus* and *Phoenix dactylifera* showed the presence of various secondary metabolites such as alkaloids, tannin, saponin, cyanogenic glycoside in both plants. However, the presence of flavonoids was only recorded for *Phoenix dactylifera*. Flavonoids and phenolics are free radical scavengers and prevent oxidative cell damage and have strong anticancer activities (Ugwu *et al*., 2013). Saponins have the property of precipitating and coagulating red blood cells (Yadav and Agarwala, 2011) and also responsible for cell growth and division and have inhibitory effect on inflammation (Okwu and Emineke, 2006). Also, the use of tannin in the treatment of inflamed or ulcerated tissues and prevention of cancer has been reported (Okwu and Emineke, 2006).

*Conclusion:* The findings in this present study revealed that titanium dioxide nanoparticles have the potential to cause negative effect on hematological parameters of male wistar rats. The study also shows that administration of C. *esculentus*, P. *dactylifera* had various levels of therapeutic ability. The synergy between the combination of C. *esculentus* + P. *dactylifera* had more ameliorative property to $TiO_2$NP than singularly.

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