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Research paper

Evaluation of the preventive effects of alligator pepper aframomum melegueta aqueous extract against titanium dioxide nanoparticles induced oxidative stress in adult wistar rats

Fatima H. Al-Asady [1] *, Mohammed W. H. AL-Mohanna [1]

[1] *Department of Biology, College of Education for Pure Sciences, Kerbala University*
**Corresponding author E-mail: almosawy2014@gmail.com*

Abstract

This study evaluated the potential protective effects of Alligator pepper Aframomum melegueta (AM) seeds' aqueous extract in adult Wistar rats treated with Titanium Dioxide Nanoparticles (Tio2NP). The study included the preparation of an A. melegueta aqueous extract of AP. Thirty-six healthy adult male Wistar rats were used, randomly distributed into six groups containing six rats, each dosed orally for 60 consecutive days; group 1: control group (5ml b.w. distilled water only: Group 2:100 mg/kg TIO2 NP suspension). Group 3:100mg/kg AM Aqueous extract). Group 4: 200 mg/kg AM Aqueous extract). Group 5:100 mg/kg b.w. AM before 4 hours receive TIO2 NP 100 mg/kg b.w.). Group 6:200 mg/kg b.w AM Aqueous extract before four h receive TIO2 NP 100 mg/kg b.w.The study results revealed that Restoring normal levels of blood lipid profile in G5andG6 is represented by a significant $p < 0.05$ decrease in the level of cholesterol, LDL accompanied by a significant $p < 0.05$ increase in the level of HDL compared to the Tio2NP group. A. melegueta aqueous extract 100 and 200 mg/kg was highly protected from the toxicity of TIO2 NP in Wistar rats by a significant $p < 0.05$ increase of SOD and Catalase level and $p < 0.05$ decreased MDA. The study suggested that when taken orally, the aqueous extract of AM seeds improves the defense against adverse effects of Tio2NP that cause oxidative stress and change the lipid profile, depending on the dose.

Keywords: *TIO2 NP Oxidative Stress; ANTIOXIDANT; Aframomum Melegueta. Lipid Profile.*

1. Introduction

Medicinal plants have been used since ancient times and throughout the ages in various civilizations and countries because of their great pharmaceutical importance in treating many medical conditions and maintaining human health because of their biological activity (Nwala et al.,2013; Omoboyowa, 2017). Among these plants is the alligator pepper Aframomum meleguta; internationally, it is called the grains of paradise. It is a member of the ginger family of deciduous herbaceous plants widespread in West Africa. Locally, it is known by different names in several regions of Africa, such as Igbo Atare and Citta (Onoja et al., 2014; Nosiri et al., 2017). Residents use it widely in popular dishes as a spice because of its reddish-brown seeds, which have a spicy taste and aromatic smell, which add distinctive flavors to the food, such as soup, which contains fish, cow, or chicken meat, etc. (Inegbenebor et al.,2009). In addition, Alligator pepper is one of the ancient medicinal plants known among the local population because of its great medicinal properties in treating many different health conditions (Adefegha et al.,2016). As an antidote to some types of infections, it enhances sexual performance and fertility (François,2019); it has antibacterial properties (Odetunde et al., 2015), radioprotective on the liver (Nwozo et al.,2013), As a memory enhancer (Ishola et al.,2016), anti-diabetic(Ojo et al.,2021), It has antihypertensive activity (Lawal et al.,2007), And a stimulant for the immune system(Nawarinna et al.,2023). These medicinal and pharmacological properties are due to bioactive compounds with medicinal and health benefits (Olunkwa et al., 2023).

In recent years, nanotechnology has been widely used in various fields, including industrial, cosmetic, medical, biological, and energy, due to its distinctive chemical, physical, and biological properties. Among these nanomaterials that have increased in the spread and widespread use in the last decade are nanosized titanium dioxide nanoparticles (Tio2NP), which are used in various fields, depending on the size of their particles. As a bleaching agent in the production of paints, sunscreens, and cosmetics (Weir et al.,2012), It is also widely used as a food additive171. The (EFSA(2021) demonstrates that using TiO2 as an additive is no longer safe. It is used in dietary supplements, drug packaging materials, and pharmaceutical preparations(Hansen et al., 2015). Therefore, repeated use of products containing TiO2 NP increases the rate of its accumulation in human tissues and internal organs (Lee et al., 2019). Previous studies have shown that the accumulation of TiO2 NPs in tissues leads to histological and physiological changes (Valentini et al.,2019; Modrzynska et al.,2020), Which leads to oxidative stress and the production of reactive oxygen groups (Kang et al., 2008; Barnard, 2010). This leads to the stimulation of toxicity

and inflammatory responses in many organs, including tissue damage to the brain and testicles (Hong& Zhang, 2016), liver and kidneys (Meena& Paulraj,2012), And the spleen(Heringa et al.,2018), myocardial injury and vascular dilatation (Hong et al.,2014).
The present study aims to investigate the preventive effect of the aqueous extract of A. melegueta on the Tio2Np-induced Oxidative Stress and its administration in rats.

2. Materials and methods

Alligator pepper(AP) A. melegueta seeds were air-dried and ground to powder using a grinding machine. 230 g of the powder was dissolved in 500 mL of distilled water for about 24 h and then filtered. The filter was allowed to dry in an oven at 40 C. The extract was frozen at −40 ◦C and was later powdered and dissolved with 100 distilled water to give the required dose (100 mg/ kg body weight, 200 mg/kg body weight), which was administered to the rats daily (Adefegha et al., 2016).
Titanium dioxide nanoparticles Tio2Np (anatase 50 nm) were prepared according to Javaheri et al. (2023), prepared by Armina Engineering Company (Iran). The suspension of TiO2-NP was prepared by dissolving 10 grams of TiO2NPs in 100 ml of distilled water and then mixing for 15 min; from this suspension, one additional diluted to the desired dose was prepared daily (100 mg /kg) of TiO2NP.
Thirty-six healthy adult Wistar male rats(190-220g body weight). The rats were reared in a standard cage system and acclimatized for ten days. The rats were randomly distributed into six groups, each consisting of six rats, each being treated orally for 60 consecutive days; Group 1: control group (5ml b.w. distilled water only: Group2:100 mg/kg TIO2 NP suspension). Group3:100mg/kg AM Aqueous extract). Group 4: 200 mg/kg AM Aqueous extract). Group5:100 mg/kg b.w. AM before 4 hours receive TIO2 NP 100 mg/kg b.w.). Group 6:200 mg/kg b.w AM Aqueous extract before four h receive TIO2 NP 100 mg/kg b.w.
At the end of the test, Serum biochemistry determination of the level of lipids (Cholesterol, LDL, and HDL)and level activity of (Super-oxide Dismutase SOD, Catalase CAT, and malondialdehyde MDA)using Lewis et al. (2006) methods.
The research results were analyzed via one-way ANOVA. Compare the groups in the SPSS program version 18.0. The difference was considered significant as a $p < 0.05$, presented as means and standard deviation.

3. Results

After TiO2 administration, an increased level of Cholesterol, LDL, the rate was observed 60 days after TiO2-treated group 2, showing a statistically significant increase ($p < 0.05$) in the level of Cholesterol, LDL, a significant decrease ($p < 0.05$in the level HDLcomparison with (G1) (Table 1). In groups 3 and 4, 100 mg/kg and 200 mg/kg AM aqueous extract were administered with a significant decrease ($p < 0.05$the level of Cholesterol and LDL and a significant increase ($p < 0.05$) in the level of HDL compared to the G1) (Table 1).
The study showed the occurrence of a significant $p < 0.05$decrease in the level of Cholesterol and LDL in the two protective doses of AM aqueous seeds extracted (100 and 200)mg/kg before being dosed with TIO2 NPs compared with TIO2 NPgroup (G2) (Table1), and the level of serum HDL significantly $p < 0.05$ increased G5 and G6 compared to the TIO2 NPs (G2).

Table 1: Effect of A. Melegueta on Lipid Profile in Wistar Male Rats

Groups	Cholesterol	LDL	HDL
G1	66.65±0.32 a	22.34±0.18 a	68.15±0.27a
G2	89.11±0.29 b	39.07±0.07 b	54.26±0.14b
G3	65.17±0.03 a	21.74±0.23 a	69.45±0.13a
G4	65.38±0.33 a	20.88±0.29 a	69.60±0.18a
G5	79.16±0.19 c	28.34±0.21 c	60.88±0.26 c
G6	69.93±0.24 d	24.23±0.27 d	66.28±0.29 d

Mean ± SD,

Our current study showed a significant $p < 0.05$ decrease in the level of SOD and CAT in G2 treated with TIO2 NPs oral comparison with control (G1) (Table 2) and a significant $p < 0.05$ increase in the Level of (MDA) in G2 treated with TIO2 NPs oral comparison with control (G1) (Table 2), Also noted a significant $p < 0.05$ increase in the level of (SOD)and (CAT) and a significant $p < 0.05$ decrease in the level of MDA in groups 5and6, the two protective doses of AM aqueous seeds extracted (100 and 200)mg/kg before being dosed with TIO2 NPs compared with TIO2 NPgroup (G2) (Table 2).
AM aqueous extract was administered in groups 3 and 4 (100 and 200 mg/kg). It was observed that there was a significant $p < 0.05$ increase in the level of (SOD)and (CAT) and a significant $p < 0.05$ decrease in the level of MDA compared to the control (G1) (Table 2). The study's results indicated that the aqueous seed extract of A. melegueta protects from toxic effects from administering TIO2 NPs.

Table 2: Effect of A. Melegueta on the Antioxidant System in Wistar Male Rats

Groups	Sod	CAt	MDA
G1	14.67±0.16 a	17.91±0.29 a	15.59±0.19 a
G2	6.15±0.14b	6.32±0.1b	30.57±0.2b
G3	15.69±0.19 a	18.7±0.17 a	15.4±0.3 a
G4	15.95±0.27 a	18.88±0.25 a	14.88±0.25 a
G5	10.36±0.09 c	12. ±0.27 c	24.77±0.1c
G6	12.37±0.17d	16.26±0.33 d	24.77±0.1d

Mean ± SD,

4. Discussion

On the Preventive Effects of A. M. on TiO2NP toxicity, TiO2NP has been used to induce oxidative stress in rats by increasing the production of free radicals (González et al., 2015; Valentini et al., 2019). Our studies focused on oxidative stress because many studies confirmed that TiO2NP is mainly deposited in many organs, such as the kidney and liver (Wang et al., 2007; Warheit, Hoke, et al., 2007). This is compared with our result, indicated by the significant decrease ($p \leq 0.05$) in the activity of SOD and CAT and the concomitant increase in the levels of (MDA) in the G2-treated TiO2NP(100mg/kg) rats when compared with the G1 indicate the oxidative stress induced by TiO2NP It was reported by many researches that the accumulated TiO2NP in the kidney and liver might induce specific oxidative

impairment these results are consistent with(.Oberdorster et al., 2005; Meena & R. Paulraj (2012). Again AM was able to decrease the effect of this oxidative stress, as was indicated by the significant increase ($p \leq 0.05$) in SOD and CAT activity and decrease in the levels of(MDA) in groups (G5) & (G6) the rats treated with AMseed (100 and 200)mg/kg aqueous extract compared with the control(G1 these results are compared with (Morakinyo et al.2020;). This ameliorative effect is probably due to the AM possessing specific phytochemicals (flavonoids, glycosides, phenolic, saponins, alkaloids, tannins, and terpenoids) that have healing potential and medicinal purposes, such as antioxidants to counteract free radicals. (Ojo,et al.2021; Yu Sheng et al.2022;Mohammed et al., 2016; Oluokun et al., 2019). Phytochemicals such as Flavonoids found ubiquitously in plants and are the most common group of polyphenolic compounds, exhibit antioxidant activities due to having a chelating property, and they have Antioxidant properties to counteract oxidative damage (Asagba et al., 2019; Oguwike et al.,2020). Myhrstad et al. (2002); Okoro & Kadiri (2019) indicated that flavonoids can induce the activity of SOD and CAT; hence they can have lasting impacts on cellular function, and they might greatly benefit cells that are subjected to oxidative stress. The present study found that orally treated TiO2 NPs(100mg/kg) could affect lipid profile. The central fat in the blood is cholesterol, which circulates in the blood. It is required to develop several biomolecules and ensure the stability of cell membranes, including vitamins and hormones. Our research demonstrated that the lipid in blood was abnormal after orally treated TiO2 NPs in rats administered to 100 mg/kg TiO2 NPs daily for 60 days, were significantly elevated levels than the control group cholesterol and LDL in the blood serum, and significantly lower HDL levels than the control group these results are consistent with ((Chen et al., 2019). Previous research has indicated that changes in blood lipid levels cause many disease conditions Watts& Karpe, 2011; Tavridou et al.,2011; Wierzbicki et al .2012), such as hypertriglyceridemia, metabolic syndrome, cardiovascular disease (Reiner, 2017; Miller et al., 2011)was also closely related to body weight(Antoni et al., 2018). As a result, we found that decreased levels of Cholesterol and LDL, with concomitant elevated HDL in groups (G5) and (G6), the rats treated with AM seed aqueous extract compared with the control(G1 these results are consistent with (Sodipe et al., 2019). Olunkwa et al.(2023) reported that this improvement effect is probably due to critical bioactive components, which thus possess valuable pharmacological health benefits. Ghorbani (2017) suggests that plant flavonoids have beneficial effects, such as improving antioxidant and lipid profiles.

5. Conclusion

In conclusion, this study shows that the A. melegueta aqueous seed extract protects against titanium dioxide nanoparticles(Tio2NPs) that change lipid profile and induce oxidative stress; the protective effect of A. melegueta can be attributed to the phytochemical components present in the seeds of A. melegueta.

References

[1] Adefegha, S. A., Oboh, G., Adefegha, O. M., & Henle, T. (2016). Alligator pepper/Grain of paradise (Aframomum melegueta) modulates angiotensin-I converting enzyme activity, lipid profile, and oxidative imbalances in a rat model of hypercholesterolemia. Pathophysiology, 23(3), 191-202. https://doi.org/10.1016/j.pathophys.2016.05.005.

[2] Adefegha, S. A., Oboh, G., Adefegha, O. M., & Henle, T. (2016). Alligator pepper/Grain of paradise (Aframomum melegueta) modulates angiotensin-I converting enzyme activity, lipid profile, and oxidative imbalances in a rat model of hypercholesterolemia. Pathophysiology, 23(3), 191-202. https://doi.org/10.1016/j.pathophys.2016.05.005.

[3] Barnard, A. S. (2010). One-to-one comparison of sunscreen efficacy, aesthetics, and potential nanotoxicity. Nature Nanotechnology, 5(4), 271-274 https://doi.org/10.1038/nnano.2010.25.

[4] Chen, Z., Han, S., Zheng, P., Zhou, D., Zhou, S., & Jia, G. (2020). Effect of oral exposure to titanium dioxide nanoparticles on lipid metabolism in Sprague-Dawley rats. Nanoscale, 12(10), 5973-5986 https://doi.org/10.1039/C9NR10947A.

[5] EFSA Panel on Food Additives and Flavourings (FAF), Younes, M., Aquilina, G., Castle, L., Engel, K. H., Fowler, P., ... & Wright, M. (2021). Safety assessment of titanium dioxide (E171) as a food additive. Efsa Journal, 19(5), e06585

[6] François Xavier, K. N., Patrick Brice, D. D., Modeste, W. N., Esther, N., Albert, K., Pierre, K., & Pierre, W. (2019). Preventive effects of Aframomum melegueta extracts on the reproductive complications of propylthiouracil-induced hypothyroidism in male rats. Andrologia, 51(7), e13306 https://doi.org/10.1111/and.13306.

[7] Ghorbani, A. (2017). Mechanisms of antidiabetic effects of flavonoid rutin. Biomed Pharmacother.; 96:305-312 https://doi.org/10.1016/j.biopha.2017.10.001.

[8] González-Esquivel, A. E., Charles-Niño, C. L., Pacheco-Moisés, F. P., Ortiz, G. G., Jaramillo-Juárez, F., & Rincón-Sánchez, A. R. (2015). Beneficial effects of quercetin on oxidative stress in liver and kidney induced by titanium dioxide (TiO2) nanoparticles in rats. Toxicology mechanisms and methods, 25(3), 166-175. https://doi.org/10.3109/15376516.2015.1006491.

[9] Hansen, S. F., Heggelund, L. R., Besora, P. R., Mackevica, A., Boldrin, A., & Baun, A. (2016). Nanoproducts–what is actually available to European consumers? Environmental Science: Nano, 3(1), 169-180 https://doi.org/10.1039/C5EN00182J.

[10] Heringa, M. B., Peters, R. J. B., Bleys, R. L. A. W., Van Der Lee, M. K., Tromp, P. C., Van Kesteren, P. C. E., ... & Bouwmeester, H. (2018). Detection of titanium particles in human liver and spleen and possible health implications. Particle and fiber toxicology, 15, 1-9 https://doi.org/10.1186/s12989-018-0251-7.

[11] Hong, J., & Zhang, Y. Q. (2016). Murine liver damage caused by exposure to nano-titanium dioxide. Nanotechnology, 27(11), 112001 https://doi.org/10.1088/0957-4484/27/11/112001.

[12] Hong, J., Wang, L., Zhao, X., Yu, X., Sheng, L., Xu, B., ... & Hong, F. (2014). Th2 factors may be involved in TiO2 NP-induced hepatic inflammation. Journal of agricultural and food chemistry, 62(28), 6871-6878. https://doi.org/10.1021/jf501428w.

[13] Inegbenebor, U., Ebomoyi, M. I., Onyia, K. A., Amadi, K., & Aigbiremolen, A. E. (2009). Effect of alligator pepper (Zingiberaceae Aframomum melegueta) on first-trimester pregnancy in Sprague Dawley rats. Nigerian journal of physiological sciences, 24(2). https://doi.org/10.4314/njps.v24i2.52901.

[14] Ishola, I. O., Awoyemi, A. A., & Afolayan, G. O. (2016). Involvement of antioxidant system in the amelioration of scopolamine-induced memory impairment by grains of paradise (Aframomum melegueta K. Schum.) extract. Drug Research, 455-463 https://doi.org/10.1055/s-0042-109391.

[15] Javaheri, R., Raji, A. R., Jafari, A. M., & Nourani, H. (2023). Effects of Oral Exposure to Titanium Dioxide Nanoparticles on the Liver, Small Intestine, and Kidney of Rats assessed by light microscopy and Transmission Electron Microscopy. Iranian Journal of Veterinary Science & Technology, 15(1). https://doi.org/10.1007/978-981-19-6845-7_1.

[16] Kang, S. J., Kim, B. M., Lee, Y. J., & Chung, H. W. (2008). Titanium dioxide nanoparticles trigger p53-mediated damage response in peripheral blood lymphocytes. Environmental and molecular mutagenesis, 49(5), 399-405. https://doi.org/10.1002/em.20399.

[17] Lawal, B. A. S., Aderibigbe, A. O., Essiet, G. A., & Essien, A. D. (2007). Hypotensive and antihypertensive effects of Aframomum melegueta seeds in humans

[18] Lee, J., Jeong, J. S., Kim, S. Y., Park, M. K., Choi, S. D., Kim, U. J., ... & Yu, W. J. (2019). Titanium dioxide nanoparticles oral exposure to pregnant rats and its distribution. Particle and fiber toxicology, 16, 1–12. https://doi.org/10.1186/s12989-019-0313-5.

[19] Lewis,S.M.;Bain,B.J.andBates,I.(2006).Dacie and Lewis practical heamatology ,10th edn., Elsevier, Germany: 709.

[20] Meena, R., & Paulraj, R. (2012). Oxidative stress-mediated cytotoxicity of TiO2 nano anatase in liver and kidney of Wistar rat. Toxicological & Environmental Chemistry, 94(1), 146-163 https://doi.org/10.1080/02772248.2011.638441.

[21] Miller, M., Stone, N. J., Ballantyne, C., Bittner, V., Criqui, M. H., Ginsberg, H. N., ... & Pennathur, S. (2011). Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation, 123(20), 2292–2333. https://doi.org/10.1161/CIR.0b013e3182160726.

[22] Modrzynska, J., Mortensen, A., Berthing, T., Ravn-Haren, G., Szarek, J., Saber, A. T., & Vogel, U. (2021). Effect on mouse liver morphology of CeO2, TiO2, and carbon black nanoparticles translocated from lungs or deposited intravenously. Applied Nano, 2(3), 222-241 https://doi.org/10.3390/applnano2030016.

[23] Mohammed, A., Koorbanally, N. A., & Islam, M. S. (2016). Phytochemistry, antioxidative activity, and inhibition of key enzymes linked to type 2 diabetes by various parts of Aframomum melegueta in vitro. Acta Pol Pharm, 73(2), 403-417.

[24] Morakinyo, A. E., Fasakin, W. O., Fajobi, A. O., Akinpelu, B. A., & Oyedapo, O. O. EFFECTS OF AFRAMOMUM MELEGUETA LEAF EXTRACT ON THE ANTIOXIDANT STATUS OF OBESE RATS.

[25] Morakinyo, A. E., Oyedepo, T. A., Babarinde, O., Adelowo, J. M., Adepoju, A. E., & Oyedapo, O. O. (2019). Anti-hyperglycemic and anti-hyperlipidemic potentials of methanol leaf extracts of Aframomum melegueta and Piper guineense. Eur. J. Med. Chem. Plants, 1-8. https://doi.org/10.9734/ejmp/2019/v27i130106.

[26] Nosiri, C., Anyanwu, C., Agim, C., & Nwaogwugwu, C. (2017). Evaluating Aframomum melegueta aqueous seed extract on food and water consumption in albino rats. IOSR Journal Of Pharmacy. Volume, 7, 23-26. https://doi.org/10.9790/3013-0704012326.

[27] Nwala, C. O., Akaninwor, J. O., & Monanu, M. O. (2013). Phytochemical screening and wound healing activities of extracts of Jatropha curcas leaf formulated in a simple ointment base. International Journal of Engineering Science Invention, 2(6), 72-75

[28] Nwarienne, C. M., Igbokwe, G. E., Ikwuka, D. C., Ezugwu, U. M., & Ngobidi, K. C. (2023). Effects of Zingiber officinale and Seeds of Aframomum melegueta Extract on Some Biochemical and Immunological Indices of Electric Foot Shock Stress-Induced Wistar Rats. Journal of Advances in Medical and Pharmaceutical Sciences, 25(8), 25-37 https://doi.org/10.9734/jamps/2023/v25i8634.

[29] Oberdorster, G., E. Oberdorster, and J. Oberdorster. 2005. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environmental Health Perspectives 113:823–39 https://doi.org/10.1289/ehp.7339.

[30] Odetunde, S. K., Adekola, I. T., Avungbeto, M. O., & Lawal, A. K. (2015). Antimicrobial effect and phytochemical analysis of Aframomum melegueta on some selected bacteria and fungi. European Journal of Biotechnology and Bioscience, 3(4), 15-19

[31] Oguwike, FN; Offor, CC; Ughachukwu, P; Chukwu, LC; Okafor, IJ; Emenuga, V; Ezenwa, SC (2020). Effect of Aqueous Extract of Aframomum melegueta (Alligator Pepper) on some Haematological and Biochemical Profile of Albino Wistar rat. Greener Journal of Medical Sciences, 10(1): 1-6.

[32] Ojo, O. A., Ojo, A. B., Okolie, C., Abdurrahman, J., Barnabas, M., Evbuomwan, I. O., ... & Batiha, G. E. S. (2021). Elucidating the interactions of compounds identified from Aframomum melegueta seeds as promising candidates for the management of diabetes mellitus: A computational approach. Informatics in Medicine Unlocked, 26, 100720. https://doi.org/10.1016/j.imu.2021.100720.

[33] Ojo, O. A., Ojo, A. B., Okolie, C., Abdurrahman, J., Barnabas, M., Evbuomwan, I. O., ... & Batiha, G. E. S. (2021). Elucidating the interactions of compounds identified from Aframomum melegueta seeds as promising candidates for the management of diabetes mellitus: A computational approach. Informatics in Medicine Unlocked, 26, 100720. https://doi.org/10.1016/j.imu.2021.100720.

[34] Olunkwa, U. E., Iheanacho, K. M. E., Igwe, C. U., Nwaogu, L. A., & Iheanacho, J. N. (2023). Bioactive component analysis of aqueous seed extract of Aframomum malagueta. GSC Biological and Pharmaceutical Sciences, 25(2), 249-272 https://doi.org/10.30574/gscbps.2023.25.2.0458.

[35] Omoboyowa, D. A., Aja, A. O., Eluu, F., & Ngobidi, K. C. (2017). Effects of Methanol Seed Extract of Aframomum melegueta (Alligator Pepper) on Wistar Rats with 2, 4-Dinitrophenylhydrazine-induced Hemolytic Anemia. Recent Advances in Biology and Medicine. https://doi.org/10.18639/RABM.2017.03.443648.

[36] Onoja, S. O., Omeh, Y. N., Ezeja, M. I., & Chukwu, M. N. (2014). Evaluation of the in vitro and in vivo antioxidant potentials of Aframomum melegueta methanolic seed extract. Journal of Tropical Medicine, 2014. https://doi.org/10.1155/2014/159343.

[37] R. Meena & R. Paulraj (2012): Oxidative stress mediated cytotoxicity of TiO2 nano anatase in liver and kidney of Wistar rat, Toxicological & Environmental Chemistry, 94:1,146-163 https://doi.org/10.1080/02772248.2011.638441.

[38] Reiner, Ž. (2017). Hypertriglyceridaemia and risk of coronary artery disease. Nature Reviews Cardiology, 14(7), 401–411. https://doi.org/10.1038/nrcardio.2017.31.

[39] Sodipe, O. G., Ajayi, O. A., Agbo, S. O., Iyeh, C. I., Abioja, M. O., Osunkeye, O. J., & Osinowo, O. A. (2019). Effects of Aframomum Melegueta Seed Extract on the Haematological and some Biochemical Parameters in Albino Rats. FUOYE Journal of Pure and Applied Sciences (FJPAS), 3(1), 122–129.

[40] Tavridou, A., Ragia, G., & G Manolopoulos, V. (2011). Emerging targets for the treatment of dyslipidemia. Current medicinal chemistry, 18(6), 909-922. https://doi.org/10.2174/092986711794927757.

[41] Valentini, X., Rugira, P., Frau, A., Tagliatti, V., Conotte, R., Laurent, S., ... & Nonclercq, D. (2019). Hepatic and renal toxicity induced by TiO 2 nanoparticles in rats: a morphological and metabonomic study. Journal of Toxicology, 2019. https://doi.org/10.1155/2019/5767012.

[42] Valentini, X., Rugira, P., Frau, A., Tagliatti, V., Conotte, R., Laurent, S., ... & Nonclercq, D. (2019). Hepatic and renal toxicity induced by TiO 2 nanoparticles in rats: a morphological and metabonomic study. Journal of Toxicology, 2019 https://doi.org/10.1155/2019/5767012.

[43] Wang, J.X., G.Q. Zhou, C.Y. Chen, H.W. Yu, T.C. Wang, and Y.M. Ma. 2007. Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration.Toxicology Letters 168: 176–85 https://doi.org/10.1016/j.toxlet.2006.12.001.

[44] Warheit, D.B., R.A. Hoke, C. Finlay, E.M. Donner, K.L. Reed, and C.M. Sayes. 2007.Development of a base of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicology Letters 171: 99–110 https://doi.org/10.1016/j.toxlet.2007.04.008.

[45] Watts, G. F., & Karpe, F. (2011). Triglycerides and atherogenic dyslipidaemia: extending treatment beyond statins in the high-risk cardiovascular patient. Heart, 97(5), 350-356. https://doi.org/10.1136/hrt.2010.204990.

[46] Weir, A., Westerhoff, P., Fabricius, L., Hristovski, K., & Von Goetz, N. (2012). Titanium dioxide nanoparticles in food and personal care products. Environmental science & technology, 46(4), 2242-2250 https://doi.org/10.1021/es204168d.

[47] Wierzbicki, A. S., Hardman, T. C., & Viljoen, A. (2012). New lipid-lowering drugs: an update. International journal of clinical practice, 66(3), 270-280. https://doi.org/10.1111/j.1742-1241.2011.02867.x.

[48] Yu Sheng Toh, E., Lim, C. L., Pick Kiong Ling, A., Chye, S. M., & Koh, R. Y. (2019). Overview of the Pharmacological Activities of Aframomum melegueta. Pertanika Journal of Tropical Agricultural Science, 42(1).