Cellular Total Lipid Peroxidation, and Glutathione S Transferase Levels in Larvae and Pupae of Aedes Aegypti with Catalysts Preparation of Mg-doped TiO2 Nanoparticles.

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Abstract. Aim: synthesis, characterization, and application of modifying nanocomposite TiO2 doped with Magnesium for photodegradation of antioxidant system Larvae and Pupae of Aedes Aegypti Catalysts Preparation of Mg-doped TiO2 to determine activity of oxidative stress (MDA) and glutathione S Transferase, were known as a parameter of defense system resistance and immune maintained. This study was undertaken to assess the potential role of growth of stages of Aedes Aegypti correspondence with oxidant and antioxidant balance triggered by nanoparticle exposure. The amounts of these parameters in cellular samples were investigated using the following materials and procedures, intake 100 larvae and 100 pupae as subjects with (study subjects) and 3-9 days’ age-matched with healthy subjects as controls. at the second of the admission, as a marker of lipid peroxidation, and therefore an indicator of the activity of standard free radicals Nanoparticles Photo Catalysts, TiO2 doped with Mg, the standard prepared Nanopowder changes from the forbidden band TiO 2 standard doping with atoms of Mg (Mg) using the sol-gel method, for Mg-doped TiO2 nanoparticles, the estimated band gap energy is 2.92 eV. Tissue MDA was used to estimate thiobarbituric acid reactive substances (TBARS), and liquid glutathione reductase activity was assessed using Goldberg DM's method. Results: When compared to controls, there was a dramatic rise in MDA content and glutathione s transferase efficiency in larvae and pupae populations exposed to photo catalyst modified nanoparticles. Conclusion: Increased MDA support to oxidative stress in larvae and pupae samples supports enhanced oxygen-free radical generation, as indicated by our findings. Increased antioxidant enzyme activity could be a compensatory mechanism in response to increased oxidative stress. The findings point to glutathione s transferase's antioxidant activity in response to increasing oxidative stress in the treated group.

Keywords: Titanium Dioxide, Bandgap energy, Aedes Aegypti, malondialdehyde (MDA), glutathione s transferase
Introduction

The bite of a mosquito infected with the aedes species transmits dengue infections into people (ae. aegypti or ae. albopictus). In more than 100 countries worldwide, dengue fever is common. 40% of the world's population, or approximately 3 billion, lives in dengue-prone areas. Dengue fever is often a major cause of disease in evocative areas (1,3). Adopting a strategy to control this mosquito by investigating the weakest links in its life cycle, with a significant increase in casualties reaching the cold regions of southern Europe and elsewhere, with the phenomenon of global warming spreading in both developed and developing countries. Aedes aegypti and Aedes albopictus mosquito life stages (4,5) eggs. Adult female mosquitoes lay their eggs on the walls inside containers with water, above the line where it reaches water. Eggs adhere to the walls of the container as if they had glue. They can survive drying for up to 8 months, mosquito eggs can even survive a winter in the southern United States. Mosquitoes, on the other hand, simply require a minimal amount of water to deposit their eggs. Bowls, cups, platters, tires, barrels, vases, and any other water-holding container are ideal "hatchery" materials. (5,8). Larvae, or larvae, are creatures that live in water. Mosquito eggs can even survive a winter in the southern United States. Mosquitoes, on the other hand, simply require a minimal amount of water to deposit their eggs. Bowls, cups, platters, tires, barrels, vases, and any other water-holding container are ideal "hatchery" materials. (5,8). Larvae, or larvae, are creatures that live in water. Mosquito eggs are the source of these pests. When water (from rain or irrigation) covers the eggs, the larvae can be seen swimming around in the water. They are incredibly busy and are frequently referred to as "worms." Pupae (or chrysalis) reside in water, and an adult mosquito emerges from the pupa and flies. Adult female mosquitoes • Adult female mosquitoes bite humans and animals. • Female mosquitoes look for sources of water to lay their eggs after feeding, and they need blood to do so. Aedes aegypti and Aedes albopictus flying large distances is not recommended. A mosquito will only fly a few blocks away for the rest of its life; Aedes aegypti mosquitoes prefer to reside near people to avoid the itch, but Aedes albopictus mosquitoes bite people and animals and can live inside or near houses. mosquitoes can be found both indoors and outside. Nanoparticles are small particles with a toxic effect., Aedes aegypti (diptera: culicidae) (9-14) has been accustomed to urban areas. the process of urbanization creates favorable habitats for this disease vector, increasing the likelihood of pathogen transmission in high-density areas. metal-stressed larval habitats can be found in city contexts. however, little is known about the physiological cost of oxidative stress or how it may influence mosquito function. the goal of this research is to determine the physiological implications of oxidative stress in Aedes aegypti that are fatal and sub-lethal (15-18) under larval exposure stress, several aspects of mosquito physiology are investigated, including larval biochemical expression and the consequences of larval stress on adult performance and progeny. environmentally related larval metal stress impairs larval and adult development and performance, as well as causing larval metal tolerance and increased lipid intake, according to the findings. a considerable increase in enzyme and co-enzyme-like glutathione s transferase expression in body tissue is associated with these Aedes aegypti performance costs. In compared to non-metal-stressed individuals, oxidative stress causes a reduction in body weight and allows balanced reserve lipid to be released when they emerge (MDA)(19-24), starvation tolerances, fertility, and starvation tolerance. Ironically speaking, larval exposure leads to a reduced life span for the adult after 32-72 hours. These findings together reveal that, although the larval oxidative stress is low, it has significant physiological implications for these important infections.

Refers to a group of abnormality pathophysiological status caused by overproduction of reactive oxygen species (ROS) are mutagenic and are persistently upregulated in Aedes aegypti that cause break growth or death, swelling in the chest region, and loss of motion I which undergoes the NADPH Oxidase System Oxidative Blast for the production of superoxide (O2•-) that accompanies the consumption of oxygen. The superoxide produced will then be converted into H2O2 and other oxidants that cause oxidation and damage. To lipids, proteins, and DNA in
exposed tissue. In an acid environment, nps dissolve to release iron particles, which should kick start hydrogen peroxide and superoxide to generate (ROS) is a toxic, systemic degradation of anti-oxidant. Where different tissues were mostly inflamed in the body, resulting in the release of MDA as a final product in lipid peroxidation and possible loss of function. It is an immune abnormality in which the immune system of the body attacks itself. Nps affect approximately 90% to 80% of the total mosquito population [24-29]. Every exposed, an annual exposure rate of nanoparticles nps ranging from 30% to 40% of the total population is reported, particularly after modifying the energy gap by doping with Mg. Every year, East Asia reports a decrease in the influence of nps (30-33). Nps affect roughly one-fifth of the population, carbohydrate chains of glycoproteins and glycolipids, as well as non-reducing residues. Increased levels of GST were observed in several parameters, tests, chemical exposure resistance, and differentiation and toxic exposure, such as organophosphate abatement, nps, DDT, and infarction with inorganic insecticides. GST is also increased as a result of high concentrations of wealth during the progress of exposure processes (34-38). The useful parameter of cellular GST was reported for inflammation and tissue damage and detoxification systems for larvae by toxic exposure dysfunction (39-43). However, other investigations have shown that glutathione transferase is an effective defense molecule against oxidation and induce apoptosis. Caused by H2O2 (43-46). However, its importance in various pathological conditions has not been discussed. Based on the pivotal mechanism for the progression of Mg-doped tio2 exposure, GST levels and oxidant-antioxidant status of oxidant damage caused by free radicals have been assessed. Free radical-Influenced peroxidation of lipids is seen as an important mechanism of the destruction of cellular membranes and cell destruction. The physiological and pathological establishment by free radicals occurs in the mosquito tissues. (47) A significant element in tissue damage induced by several pathophysiology is the uncontrolled manufacture of free radicals (48-50). Alterations in the oxidative-antioxidant profile are known in larvae and pupae of Aedes Aegypti with-doped-tio2 catalysts preparation as a result of energy gap changes. Free radicals are known to affect the reaction of these people to exposure by oxidizing stress. Moreover, the body’s stress responses, in the form of antioxidants, wanting to minimize damage while adapting to the above stressful situation would play an important role. Antioxidants are compounds which dispose of free radicals, scavenge and suppress individuals, or oppose them. (39,40). Antioxidants are divided into two categories: those that prevent free radical generation and those that intercept any free radicals that are created. (50-53) They might be enzymes or non-enzymes and can be present in both the aqueous and membrane compartments of cells (54,55). Our study focused on and on the effectiveness and change in the status and relationship of oxidant and antioxidant in larvae and pupae of mosquitoes at different Mg-Doped tio2 concentrations.

Materials and methods

The study was conducted in the Department of Biochemistry, and Department of Virology Veterinary Medical College & Putra University, Serdang, Selangor state, Malaysia. One hundred laboratory-diagnosed subjects of each experiment from the A study selected for the department of parasitology, which had not previously received any treatment for its chemical or toxic exposures. Pupae account for 100 of the 200 participants in the sample. A similar environment-laboratory status was also examined in an equal age and feed-healthful participants who were associated. The research group’s larvae were matched to the control group's larvae. Transitions in larvae were considered for matching because differentiation and growth hormones, in oxidant status and NPs component doses, as well as their transporters, plays a significant influence. The shape and weight of the body were also taken into account. Before the research began, approval was sought from the institution's ethical committee. Before the study, written consent was obtained from the
participant, and the study's objectives were thoroughly clarified. The subjects' entire experimental history, as well as their individual histories, were documented. The participants were 3–9 days old at the time of the study. All of the respondents were given NPs as an experimental procedure. The presence of GST and MDA status in samples was determined using a factor test that included macroscopically and biochemical examination of tissue damage, movements, and active symptoms. None of these subjects had been exposed to alcohol or chemicals and did not have any systemic disorders. The samples were removed from the analysis if they had a disease other than weakness. Subjects with regular dietary patterns who had not taken any vitamins during the previous treatment were included in the study. There were two categories of controllers and treatments.

Group 1: 100 healthy controls of similar age and growth with identical lab status.
Group 2: 100 people who were exposed to NPs that had been experimentally diagnosed.

The researchers analyzed cellular samples taken from these participants in the morning following an overnight fast. Centrifugation at 1000 g for 15 minutes at +4 °C was used to separate the samples. GST and MDA were calculated using separated cellular fluids. MDA and glutathione s transferase levels were measured using Warren's thiobarbituric acid test technique (14). The glutathione s transferase activity in the samples was evaluated using Goldberg DM's technique, and MDA was computed as a measurement of thiobarbituric acid reactive compounds (TBARS) (15). (16). All required precautions were performed throughout sample collection, storage, and evaluation.

Chemicals

The chemicals used were all reagents or the highest grade available. Sigma Chemicals is a company that manufactures chemicals, St. Louis, MO, USA, provided glutathione and thiobarbituric acid. The Mann-Whitney U test was used to compare group 1 (controls) and group 2 (treatment) statistically. The data is presented as a mean ± standard deviation. The significance level was set at mean ± SD, P < 0.05.

Analytical statistics

The Independent Student's t-test was used to conduct statistical analysis comparing groups 1 (controls) and 2 (treatments) using SPSS for Windows version 25. The data are presented as mean ± SD, with a significance level of P < 0.05.

Results, Discussion

Characterization of the photo catalyst

XRD patterns have been used to determine the crystallite size and phase composition of TiO2- and Mg-doped TiO2 nanoparticles. with 2theta diffraction angles ranging from 15 to 70°. Fig. 1 (a, b, c) summarizes the obtained results. Figure 1 illustrates the diffraction patterns of TiO2- and Mg-doped TiO2 nanoparticles calcined at 400 and 500°C. In the XRD assessment of nanoscale TiO2- and Mg-doped TiO2 calcined at 300 and 400°C, the primary crystalline peak exhibited anatase of varying intensities, as shown in the figure (1, c, d) (diffraction peak at 2 thetas = 25.3°). There is no contaminant peak that corresponds to Mg or the rutile phase. The doping method without affecting crystal structure creates high intensity and sharper anatase peaks and boosts the crystallinity of the anatase phase, according to XRD data (37). Low metal ion dopant concentration and proper metal ion deposit dispersion on TiO2 nanoparticles are responsible for
the Mg peak, (56,47). Figure 1.(c) which identify a specific the XRD pattern of Mg-doped TiO2 calcined at 500°C. The crystallinity of TiO2 particles improves as the calcination temperature rises, transforming amorphous TiO2 into anatase and ultimately rutile phases. The temperature at which the anatase phase transitions to the rutile phase is determined by the conditions under which the particles are formed, as well as their attributes with (39,56). Substantial improvements in the phase structure of Mg-doped TiO2 nanoparticles were detected when the calcination temperature was increased to 500°C. The anatase changed into a rutile phase after 500°C calcination, having a diffraction peak at 2 theta = 27.4°. Transmission electron microscopy was used to evaluate the particle size of Mg-doped TiO2 nanoparticles (Fig. 3). As can be seen, the prepared samples have agglomerated into bigger particles. The TiO2 nanoparticles' average particle size, on the other hand, was less than 20 nm, which is close to the crystallite size determined from the XRD pattern. Due to low metal content below the detection limit, analysis of 0.2 mol percent Mg-doped TiO2 revealed only Ti and O peaks. The effect of Mg addition on the bandgap energy of TiO2 was investigated using DRS. In defining TiO2 band gap energy levels, crystallite size and imperfections in the TiO2 network are critical features (40). Figure 2 displays the optical absorption of Nano TiO2 and Mg-doped TiO2 as well as the two and three diameters of Mg-TiO2. The absorption wavelength for Mg-doped TiO2 is due to the electrical transition between the defect level and the band structures of TiO2.

Fig.1. XRD (a) Pure TiO2, Mg-TiO2 and 10% Mg-TiO2 (b) (0.2% mol) Mg-TiO2 at varying temperatures of calcination (c)XRD for Mg-TiO2(d), XRD for TiO2
TiO$_2$ is significantly red-shifted compared to TiO$_2$ nanoparticles. Mg-doped TiO$_2$ nanoparticles have calculated bandgap energy of 2.82 eV. Mean ± SD of glutathione s transferase, as indicated in table 2 and figure 5, malondialdehyde (MDA) concentration and activities were measured in controls and treatments with Mg-doped TiO2 exposed. When NPs in treatment group were compared to controls, Figure 4 shows that malondialdehyde (MDA) quantities and glutathione s transferase activity both increased statistically significantly.

Table 1. TiO$_2$ and doped samples feature phase structure and crystallite size.

| Photo catalyst            | Calcination temperature (°C) | Quantity per each phase % | Dimensions of crystallites (nm) | Particle Size range(nm) | $D_a$ | L(nm) |
|---------------------------|-----------------------------|----------------------------|---------------------------------|------------------------|-------|-------|
| TiO$_2$                   | 300(°C)                     | A: 100, R: –               | $D_A$: 13, $D_R$: –             | 60-135                 | 84.4  | 26.   |
| Mg–TiO$_2$ (0.2% mol)     | 300(°C)                     | A: 100, R: –               | $D_A$: –, $D_R$: 13             | 12-118                 | 80.64 | 26.   |
| Mg–TiO$_2$ (0.2% mol)     | 400(°C)                     | A: 100, R: –               | $D_A$: –, $D_R$: 12             | 52-111                 | 79.39 | 59.   |
| Mg–TiO$_2$ (0.2% mol)     | 500(°C)                     | A: 100, R: –               | $D_A$: –, $D_R$: 13             | 44-116                 | 76.88 | 59.   |

**Fig 2.** Mg-doped TiO$_2$ by Atomic force microscopy (AFM) AFM

Fig. 3. refers to the parameter of particles Nanocomposite of The size and distribution of two-dimensional and three-dimensional particles were measured using an AA2000 atomic force microscope, and the results were summarized in table 1.

**Table 1.** TiO$_2$ and doped samples feature phase structure and crystallite size.
**Figure 3.** Micrographs of (a) Nano TiO$_2$ and (b) Mg-doped TiO$_2$ taken with a transmission electron microscope.

**Table 2.** Total lipid peroxidation, malondialdehyde (MDA), and glutathione S Transferase activity in controls and treatment groups Mean ± SD

|            | N  | Mean   | Std. D | t      | df | P value |
|------------|----|--------|--------|--------|----|---------|
| TiO2-Mg-200μM | 214 | .460898| .296   | 17.211 | 213| .000    |
| TiO2-Mg-300μM | 214 | .991132| .678   | 15.191 | 213| .000    |
| TiO2-Mg-400μM | 217 | 1.58250| .291   | 58.908 | 216| .000    |
| MDA -500 -dose -3 | 214 | 1.785914| 3.357 | 17.211 | 213| .000    |
| MDA -500 -dose -2 | 214 | 1.442628| 1.5451 | 13.659 | 213| .000    |
| MDA -500 -dose -1 | 214 | .947465| 1.171 | 11.843 | 213| .000    |

**Figure 4.** Comparative mean ± SD of GST among studied groups
Figure 5. Comparative mean and S.D. of MDA among studied groups

The lipid peroxidation product MDA is part of the overall study, was found to be significantly higher in the serum of cellular fluids treated with Mg-doped TiO$_2$. The MDA increasing trend could be attributed to the increasing production by these subjects of reactive oxygen species (ROS). Many other important biomolecules, including membrane lipids, can be oxidized by these oxygen species. MDA levels have been observed to be raised in patients treated with nanoparticles in a comparable reports pattern (11,17). In contrast to our findings, (38,39). No significant difference in MDA levels between Mg-doped TiO$_2$ treatments and controls has been found in our results which correspond with (18). hen subjects treated with nanoparticle irradiation were paralleled to controls, it’s found a significant importance in glutathione s transferase levels. Our total study shows significant increases in glutathione transferase concentrations in response to oxidative stress We therefore recommend that higher glutathione transmission levels may be considered as a defense molecule against increased oxidative stress in radiation from NPs. Tanaka et al. have reported the antioxidant property of GST as an H$_2$O$_2$ scavenger (19). The tissue enzyme, i.e. the transferase activity of glutathi one, was significantly improved in samples with NPs in our study. In treatments with Mg-doped TiO$_2$, comparable reports of increased anti-oxidants enzymes activities have been reported by Carvalho et al., Al-Salih et al., and Bautista &Burggren,(10,20,25). glutathione s transferase (GST), an oxidative stress-inducible enzyme, plays a major role in peroxyl scavenging and the maintenance of cell membranes functionally integrated this finding goes with (21). The effects of increased oxidative stress may be enhanced by GST activity as a result of its ablation. TiO$_2$- and Mg-doped TiO$_2$ nanoparticles by the sol-gel method was used to prepare various Mg contents. Acid Red 27(AR27) removal was investigated for photocatalytic activity. When compared to the diffuse reflectance spectra of bare TiO$_2$ and Mg-doped TiO$_2$, the doped sample has red shaping and minimal energy band gaps. according to the Au–Pree et al., (58). The outcomes of XRD pattern designated that anatase was The generated Nano TiO$_2$ and Mg TiO$_2$ nanoparticles' major phase calcined at 400°C, but that anastasis totally converted into a routine phase, allowing for calcination temperatures of up to 500°C this results according to Ramandi. et al., (58). Mg-doped TiO$_2$ nanoparticles in the SEM micrograms had duly signed of ingredient-gated particles and smaller sizes of the particle as reported by Miditana et a.,1 (2021) (60). BET and BJH had mesoporous figures with a limited pore distribution (1-8 nm). Increased Mg-doped TiO$_2$ photocatalytic activity was achieved by increasing the AR27 ingredient to 0.2 mol percent. The AR27 in the presence of Mg-doped TiO$_2$ nanoparticles, removal rate was affected by catalyst dosage, contaminant concentration, light intensity, and irradiation period. these findings agree with the reported by (53-60). Complete
mineral feeding can be achieved with the optimization of these parameters which can and has proven efficient in overcoming the physiological activities with which insects maneuver by masking the lipid content upon exposure to radiation and toxic factors. As well as stimulating antioxidant systems to work and detect, and then their active sites inhibit the accumulation of active oxygen and its hydrogen peroxide products.

**Conclusion**

Finally, tissue damage and cell death from exposure to TiO$_2$- and Mg-doped TiO$_2$ nanoparticles prepared by the sol-gel method can be associated with oxidative stress. The oxidant/antioxidant balance for lipid peroxidation changes, which can lead to tissue damage observed in this exposure. The results of our study suggest that the higher oxidative stress hypothesis in Mg-doped TiO$_2$ is supported by a considerable increase in malon dialdehyde levels. Increased antioxidant enzyme activities can be compensating for increasing oxidant stress. In addition, in conjunction with oxidative stress, the GST level has increased, supporting the antioxidant GST role.

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