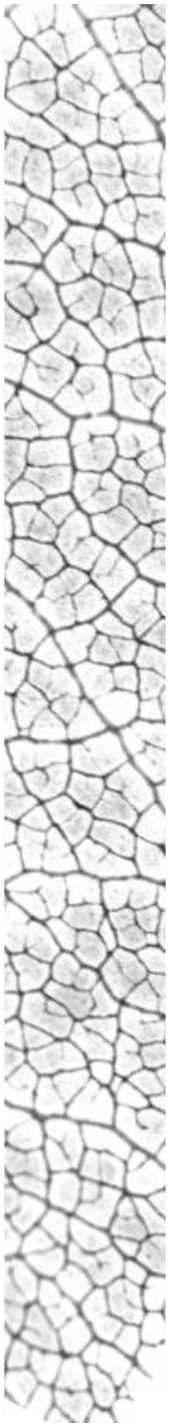

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Evaluation of cellular oxidative stress levels in aedes aegypti mosquitoes as a reaction of photo catalyst modify nanoparticles exposure

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Abstract

Objectives to estimate the cellular malondialdehyde (MDA) level– as a measure of lipid peroxidation in Aedes aegypti with exposure by Nanoparticles Photo Catalysts, Mg-doped TiO2, this study also aimed to evaluate the difference in MDA levels in each of life stage and between treatment group and control. Treatment and a case-control study including 100 adults (50 females and 50 male) 100 larva and 100 pupae Aedes aegypti whose follow-up was carried out at the Virology Lab. All the subjects were subjected to estimation malondialdehyde (MDA) at the time of admission, as a marker of lipid peroxidation, and hence an indicator of free radical activity by Nanoparticles Photo Catalysts, Mg-doped TiO2, the Nano powder prepared by modification of the TiO2 band gap by doping with Mg ،Mg) atoms using sol-gel method. XRD and AFM recognition shows clear peaks assigned to TiO2 (mainly peaks: 2theta = 45 and 52o) indicating that a Mg, ions were safely incorporated into the titanium anatase framework. All sample treated with manpower than This method is based on the principle that acetic acid detaches the lipid and protein of a tissue, thiobarbituric acid reacting with lipid peroxide, hydroperoxide, and oxygen-labile double bond to form the color adducts with maximal absorbance at 530 nm. Student 't' test was used for unpaired samples to compare the means of the control and the cases and also the various inter-group differences. Significance was accepted if the null hypothesis was rejected at $p < 0.05$. The difference in MDA levels in cases and controls was seen to be statistically significant ($p < 0.001$), suggesting an increase in the level of lipid peroxides The mean MDA level in the control population was 1.9 ± 0.4 nmol/dl (1.9 ± 0.1 in males; 1.9 ± 0.8 in females), while the corresponding value in pupae and larva groups were 4.16 ± 1.04 nmol/dl (4.01 ± 1.9 in pupae; 4.5 ± 1.1 in larva) and 4.03 ± 1.1 nmol/dl (3.76 ± 0.7 in males. In conclusion, in present found high concentrations of products derived from lipid peroxidation while assessing levels of an adult, larvae, pupae, and the oxidative damage of circulating protein according to the carbonyl content of cellular protein peroxidation. Our results suggest the association of cellular damage caused by oxygen free radicals with the pathogenesis of antioxidant system in Aedes aegypti of presented highly marked modifications related to the presence of nanoparticles oxidative stress, characterized by intense lipid and protein peroxidation and reduced antioxidant defense system of Aedes aegypti.

Keywords: aedes aegypti, Malondialdehyde (MDA, Nanoparticles, Oxidative stress

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INTRODUCTION

The use of Titanium dioxide TiO_2 is an interesting alternative in these studies, since there is the possibility of using it with sunlight radiation to promote the nanoparticles as insecticide which would reduce free radicles with high energy treatment costs. Titanium dioxide is an easy-to-handle material that is very chemically stable when compared to other catalysts, and can be found at low cost. Titanium dioxide can present three different phases of polymorphs: anatase, rutile, and brookite (João et al., 2019).

Anatase and rutile are the most commonly studied phases in the photocatalytic experiments, since anatase is the most photo catalytically active, whereas rutile is thermodynamically more stable (Henderson, 2011). Regarding brookite, few studies exist related to its surface structure characterization (Nolan et al., 2012). Titanium dioxide is a semiconductor. Thus, a suitable source of energy can promote the transfer of electrons

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from the valence band to the conduction band. The energy needed to promote the photogeneration of electron–holes pairs is usually called a band gap. If this band gap is small enough, solar energy can be used as the primary source of radiation to activate this kind of catalyst. Reversely, if the band gap is high, ultra-violet (UV) radiation shall be needed. In terms of the process, when this band gap is broken or disrupted, leading to the previously described electron transfer, the titanium dioxide can promote the degradation of contaminants from the wastewater (João et al., 2019). The photogenerated electron–hole pair will allow the oxidation of water and/or organic matter as well as the reduction of oxygen and other reactive oxidative species, leading to radical moieties that are able to oxidize pollutants at the liquid bulk (Henderson, 2011). For instance, in real wastewater, some other species are present (such as Cl−, HCO_3−, CO32−, and $SO4_2$−), which can be reduced, producing radicals that can help with the degradation process of organic contaminants (Gomes et al., 2019). The typical band-gap energy of titanium dioxide is between 3–3.2 eV. Thus, UVA radiation (wavelength <400 nm) is needed for its activation. Sunlight radiation just comprises 4–5% of UVA radiation. This means that low performance is expected when natural lights applied. Therefore, to take advantage of the remaining spectrum of sunlight radiation, the visible region, the TiO_2 band gap must be reduced.

This is usually achieved by using a suitable dopant. Doping appears as a good alternative for changing the activity of TiO_2 catalysts through the optoelectrical modification of this material by then introduction of dopants with different energy levels between the conduction and valence band (Barkul et al., 2016). The applied dopants can be metals, such as no bleand transition metals, or nonmetals (N, B, S, F, and C). The noble metals (such as Ag, Au, Pd, and Pt) present some advantages such as the possibility to absorb the visible light due to the surface plasmon resonance (Zheng et al., 2011) and (Wang et al., 2012) but the high cost associated with these materials should be considered a disadvantage. The transition metals can also enhance their photocatalytic activity. However, their leaching behavior leads to the fast deactivation of the catalyst and constitutes a second source of pollution, requiring the removal of dissolved metals from treated water (Nolan et al., 2012). Therefore, nonmetals present some advantages compared to the metal dopants. Besides the effective activation of TiO_2 in the visible spectrum of sunlight due to the narrowing of the band gap that allows avoiding the recombination phenomenon, the low cost and environmentally-friendly character of this material appear as tempting features (Gomes et al., 2017) and (Ramandi et al., 2017). As nonmetal doping elements, some anionic species such as nitrogen, carbon, sulfur, and boron have been studied due to the beneficial advantages of substituting oxygen in a TiO_2 lattice and given activity to these catalysts at visible light radiation, maintaining its maximum efficiency (Irie et al., 2003) and (Ananpattarachai et al., 2009). Among the above-mentioned nonmetals, the most suitable and commonly used is nitrogen. This element can introduce few states at the valence band edge, tuning the optical band gap and acting as superficial donors (Asahi et al., 2014). Moreover, Mg instead of O in the TiO_2 lattice allows the band gap to narrow due to the 2p states of the Mg atom mixed with O 2p states (Valentin et al., 2004). In fact, it was proved that for both the anatase and rutile phases of TiO_2, the Mg 2p states were located just above the top of the O 2p valence band, which means a red shift of the absorption band edge to the visible region (Chance & Machly, 1955). Therefore, it indicates that nitrogen is the best nonmetal dopant to TiO_2, improving the photocatalytic activity at the visible light radiation, which means a low-cost energy source for the degradation of contaminants. In this context, Mg–TiO2 catalysts are interesting in this field, and lots of information about them can be found in the literature. However, data about the main changes provided by nitrogen doping or co-doping in TiO_2 is not well established and defined. In fact, to the best of our knowledge, there is no overview on the subject. In the present study, the main objective is to critically overview the information about Mg doping and co-doping changes in the characterization analysis of the typical catalysts. Then, we can understand the advantages of using these catalysts in advanced oxidation technologies for wastewater treatment, especially in what regards emerging contaminants removal. Another feature that will be analyzed is the advantage between using powder and supported TiO_2 for wastewater treatment. Finally, future perspectives regarding this kind of catalyst will be discussed considering the wastewater treatment applications.

In acting, for example, as a defense mechanism controlled by molecular stimuli or signals against damage caused by electrons with high energy that were produced by nanoparticles. (João et al., 2019) Free radicals may also be generated by different cells in the organism when induced by exogenous sources (ionizing radiation, toxins, drugs, chemical products, environmental polluting agents, etc.), and by different pathological situations. In these cases, there may be different types of affected cell components (Henderson, 2011; Al-Seeni, et al, 2017).

Under normal circumstances, Aedes aegypti as live organisms have antioxidant defense systems consisting of nonenzymatic antioxidants - including nonprotein antioxidants with low molecular weight (beta-carotene, uric acid, and so on) - and of enzymes such as Glutathione S-transferases (GSTs), superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH- Px), and glutathione reductase (GSH-Red), which are capable of metabolizing oxygen free radicals.

Table 1. Samples classification criteria according to nanoparticles activity levels

Degree of activity	Classification	Symptomatology	Weakness case
Active with treatment	A1 A2 A3	Prest Present Present	<1 Between 2 and 4 >4
Inactive with treatment	B	Absent	Normal
Inactive without treatment	C	Absent	Normal

SOD functions as a catalyst in reactions that convert superoxide anions (O_2•) into hydrogen peroxide (H_2O_2),3 which is eliminated by catalase and glutathione peroxidase enzymes; moreover, the latter enzyme also inhibits other long-chain peroxides (Zhang et al., 2019).

Oxidative damage to essential cell components caused by oxygen free radicals is generally considered a serious mechanism in the pathogeny of inhibition of Glutathione S-transferases (GSTs) activity. Increase in the levels of various biological indicators related to oxidative damage to cells, such as rupture of membranes by lipid peroxidation, rupture of DNA chains, and alteration in the structure or function of proteins, (Zheng et al., 2011) have been demonstrated in these situations. These modifications may be directly related to routine markers of inflammatory processes (Cherng Shii et al., 2019) and (Alexander et al., 2019).

In cellular sample of Aedes aegypti, oxygen free radicals may be related to, for example, the pathogenesis of nanoparticles. Cells found in inflamed tissues after Nano exposure that's once isolated and stimulated, are capable of producing oxygen free radicals. These radicals, in the presence of lipid, DNA, protein, carbohydrate, or proteoglycan molecules can cause oxidative injury (Alexander et al., 2019) and (Ahmad, 1995) In this sense, it is also well-known that there is a relation between oxygen free radicals and damage of tissues. Hydroxyl radicals may affect the Glutathione S-transferases (GSTs), (Beauchamp et al., 1971). hypochlorous acid may affect proteoglycans, and hydrogen peroxide (H_2O_2) may inhibit their synthesis (Bradford, 1976) Intra-articular administration of H_2O_2 in female rats has induced the production of glucose oxidase enzyme, which may render severe articular lesions and necrosis of chondrocytes (Felton & Summers, 1995) The combination of increase in degradation with inhibition of synthesis favors the destruction of cell.

Oxygen free radicals are lipid peroxidation-inducing agents that cause the depletion of unsaturated fatty acids of the cell membrane, thus inducing loss of cell integrity and functional alteration of cell receptors and enzymes (Bradford, 1976) and (Chance & Machly, 1955) and (Felton & Summers, 1995) Alteration in the composition of fatty acids of the cell membrane has been reported in different mosquitos adults or larvae, such as responses in Galleria mellonella larvae and Aedes Aegypti, Peroxide and hydroxyl radicals induce peroxidation of polyunsaturated fatty acids of cell membranes that, for example, determine increase in cell rigidity and deformability in the mosquitos tissues, consequently increasing the susceptibility to lysis (Jirakanjanakit et al., 2007) and (Macoris et al., 1995).

The objective of this study was to determine characteristics of cellular markers of oxidative stress and of antioxidant enzymatic cellular in samples with Aedes aegypti.

MATERIAL AND METHODS

Samples and methods

Samples

We carried out a cross-sectional study of 100 individuals, 50 females and 50 males, aged 1.6 to 15 days (age average at 9.8±5.4 years), and with nanoparticles exposure. Adult samples presented a common characteristic (n=5) of long evolution of the exposure (varying from 1 to 4 hours)

Cellular sample of Aedes aegypti in each subgroup were classified according to the degree of nanoparticles exposure activity, based on the evidence of weakness symptoms, lethal concentrations and fecundity rate at the moment of cell sample collection (**Table 1**).

The first subgroup included 17 pa the cellular samples of Aedes aegypti tients (14 female), aged 2.6 to 18 days (age average at 8.6±0.9 days). The period of exposure evolution varied from 1 to 2 hours.

The second subgroup included 35 samples (25 female), aged 1.6 to 14.5 days (age average at 11.2±5.1 years). The period of exposure evolution varied from 1 to 3 hours.

The third systemic subgroup included 12 samples (7 female), aged 3.3 to 18 days (age average at 11.0±4.6 days). The period of disease evolution varied from 2 to 4 hours.

Experimental data, exposure activity rates, and treatment at the moment of cellular sample collection were determined for all three subgroups.

The control group included 60 out individual (38 female), aged 1.5 to 2.6 days. These out samples were being submitted to follow-up at the same laboratory for non-inflammatory symptoms. Also, most control samples were being submitted to preoperative procedures for subsequent minor surgical procedure.

Prepare of samples

Cellular sample of Aedes aegypti were collected from individuals and controls as follows: 2 ml of cellular sample of Aedes aegypti with butanol as solvent; 2 ml with 2-thiobarbituric acid (TBA); and 2 ml trichloroacetic

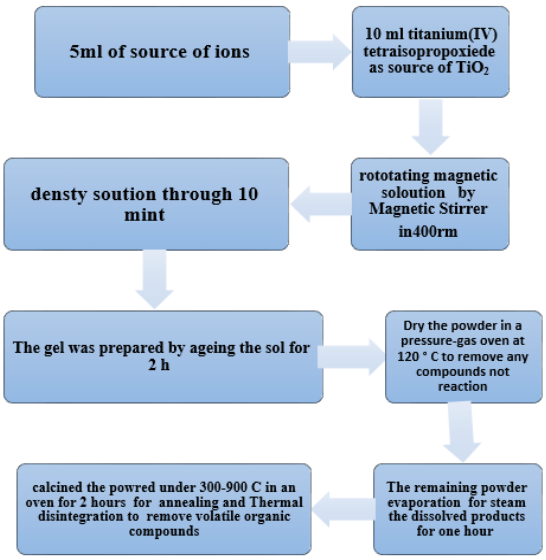

Fig. 1. Prepare modified titanium dioxide doping with Mg ions

acid (TCA) without anticoagulant. Samples aliquots were obtained after centrifuging sample.

Doping of TiO_2 and Diagnosis of Prepared Particles

Series of experimental work have been achieved to prepare modified titanium dioxide by reduction step-in solid-state reaction with A variety of different solutions contain elements atoms by serial steps as shows in (**Fig. 2**). this modify the band gap by adding different weight of each to prepared TiO_2 which the solvent to prepare the sol mixture as illustrated as flowing: - Catalysts Preparation of Mg -doped TiO_2.

Markers of lipid and protein peroxidation

We determined levels of Glutathione S-transferases (GSTs), malondialdehyde (MDA), lipoperoxide (LPO), hydroperoxide (HPX), and of carbonyl groups in tissues proteins. MDA and LPO cellular sample of Aedes aegypti levels were determined using spectrofluorometer after a reaction with diethylthiobarbituric acid (Selvaraj et al., 2013) and (Caratto et al., 2012) cellular HPX were quantified using PIERCE spectrophotometric assay (PeroXOquantTM Quantitative Peroxide Assay), according to the method described by Nourooz-Zadeh et al. Carbonyl groups of cellular proteins were measured according to method described by (Han-Dong & Qi-Zhi, 2012).

Markers of enzymatic antioxidant system activity

We determined the activity of SOD, GSH-Px, and GSH- Red enzymes, and also the levels of glutathione (GSH) using whole body samples. Enzyme activity of SOD was determined by the spectrophotometric assay, as described by (Cherng Shii et al., 2019). Activity of GST was quantified by the colorimetric assay, as described by Paglia and Valentine (Alexander et al., 2019). Activity of GSH-Red in body fluids was determined using the spectrophotometric assay, as described by Goldberg and Spooner (Ahmad, 1995), GSH was quantified using the colorimetric assay with Bioxitec TM GSH-400 reactive kit (Oxis Internation Inc., Portland, USA).

Statistical Analysis

Results were presented in typical value ± standard error for the whole group of samples, both for the clinical subgroups and for the control group. Results were compared by simple linear regression and analysis of variance (ANOVA), using the statistical software Stat View IITM.

RESULTS

In this study shows the effects of Mg–TiO_2 catalysts are interesting in this field, Therefore, it indicates that

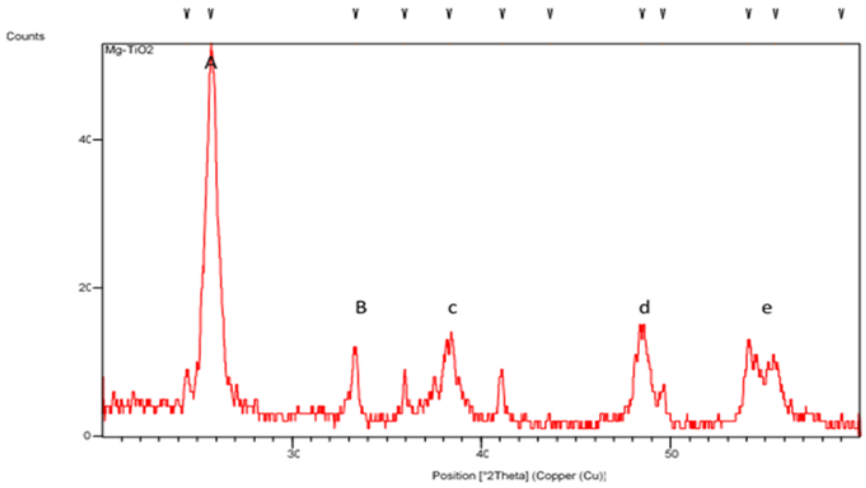

Fig. 2. X-ray diffractogram of Mg -TiO2 (Anatase) at 500Ċ

Table 2. XRD data for prepared TiO2 and TiO2 - solid state reaction

Sample TYPE	T (Ċ)	compound	Miller indices (hkl)	2Theta (deg)	High	FWHM (cm-1)	Crystal Size (nm)
TiO_2 anatase	500	TiO_2	110,101,200,111,211	25.3455	402.87	0.0720	3.51121
$MgTiO_2$	500	TiO_2	110,101,200,111,211	25.6945	47.27	0.2362	3.46718

Table 3. Markers of protein and lipid peroxidation in Aedes aegypti, in experimental subgroups, and in controls

Row	(n=64)	First (n=17)	Second (n=35)	Third (n=12)	Controls (n=60)
MDA	0.72±0.05	0.62±0.04	0.74±0.07	0.77±0.13	0.33±0.008
(M/L)	P<0.0001	P<0.0001	P<0.0001	P<0.0001	
LPO	1.92±0.08	2.05±0.17	1.77±0.1	2.21±0.15	1.76±0.05
(□ M/L)	NS	NS	NS	P<0.01	
HPX	6.04±0.83	4.75±1.04	5.40±1.03	9.75±2.78	4.76±0.53
(□ M/L)	NS	NS	NS	P<0.01	
CG	0.68±0.05	0.60±0.08	0.67±0.06	0.83±0.15	0.36±0.03
(nmol/g)	P<0.0001	P<0.01	P<0.0001	P<0.0001	

Cellular sample of Aedes aegypti; MDA: malondialdehyde; LPO: lipoperoxides; HPX: hydroperoxides; CG: protein carbonyl groups; NS: nonsignificant, significance (P) in relation to control group

nitrogen is the best nonmetal dopant to TiO_2, improving the photocatalytic activity at the visible light radiation The formation of free radicals, which is secondary to the production of reactive oxygen species, is part of the physiological process of nanoparticles exposure. In this manner, cellular metabolism produces free radicals in pathophysiology and physiological conditions. These active radicals, in turn, can be very useful.

X-Ray Diffraction (XRD) Investigation

The crystalline structure for any material can be recognized by studying the phase of that material by (XRD), when a mono wave length beam of (XRD) from system incident on film surface as shows in (Table 2), this will exhibit peaks on limited angels for each material because of reflecting of Bragg on parallel crystalline surface. XRD instrument is of type (Shimadzu 6000) made in Japan, with the following specifications. This system is available in the Center Nanotechnology / University of UKM.

Anatase characterization of standard nanoparticles TiO2 calcinated at 500oC for 2 h is shown in **Fig. 2**. The cross-section and the surface area TiO2 film indicate the film thickness and particle size 18 nm (18-20 nm) (BET), pore size 16-18 nm average pore size (BET) The porous structure of TiO2 surface will cause the large amount of dyes absorbed. Additionally, the solid surface of prepared TiO2 was enhanced of XRD pathways XRD patterns (3.5) shows that the four synthesized samples have the highest diffraction peak in crystal plane(A) (2θ = 25.3455), intensity 100% and the other diffraction peaks are consistent with crystalline phases of (004), (200), (105), (211), and (204). It can be evidently seen that the crystal phase of each and followed by (B) (101) at 2θ=37.8298˚ by intensity 25 % and (C) (111) at diffractogram angle peak 2θ=55.1004 beside to (D) (211) at diffractogram angle peak 2θ=53.9206 ˚ ˚with smaller intensity at (200), which have significant agreement of XRD standard with characterization of standard nanoparticles by s Sigma-Aldrich, Inc. Use about Titanium dioxide nano crystalline purity >95% (anatase (XRD)).

Tables 2 and **3** present results of protein and lipid peroxidation markers and of activity of the antioxidant defense system observed in the whole group of the cellular samples of Aedes aegypti, both in the clinical subgroups and in the controls.

MDA cellular levels were significantly higher in both the whole group of cellular samples of Aedes aegypti with in experimental subgroups than those of controls (**Table 2**). Treatment subgroups presented MDA values significantly higher than those of controls and progressively higher in first subgroup, second subgroup, and third subgroups (**Fig. 1**). Cellular LPO were higher among cellular sample of Aedes aegypti, but the difference was not significantly higher when compared to controls. The third subgroup presented significantly higher values when compared to the second subgroup (P<0.02) and to the controls (P<0.01) (**Table 2**).

Average physiological levels of HPX among cellular sample of Aedes aegypti were higher than those among controls. The first subgroup presented HPX values similar to those found in the control group. There were no significant differences between cellular sample of Aedes aegypti from the first subgroup and the second subgroups in relation to controls (Table 2). The third subgroup presented the highest average levels, and differences were significant when compared to the first subgroup and the second subgroups and to controls (P<0.02).

Cellular sample of Aedes aegypti protein points of carbonyl groups in the whole group of samples with Aedes aegypti and in experimental subgroups were higher when compared to controls (**Fig. 3**). Differences were statistically significant in comparing treatment (P<0.0001), second subgroup (P<0.0001), third subgroup (P<0.0001), and first subgroup (P<0.01) to the control group (**Table 2**). The highest levels of cellular protein of carbonyl groups in Aedes were observed among studied group (**Table 3**).

Average values of body GSH were significantly lower in the whole group of Aedes aegypti and in the second and the third subgroups when compared to controls

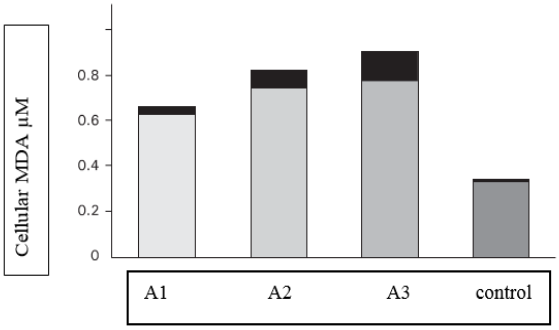

Fig. 3. Concentrations of MDA in the cellular samples of Aedes aegypti divided according to the type of treatment groups and in controls. The histograms indicate mean values, and the gray areas indicate the standard error. The level of significance of the first, second, and third sub groups, in comparison to controls, was P<0.0001

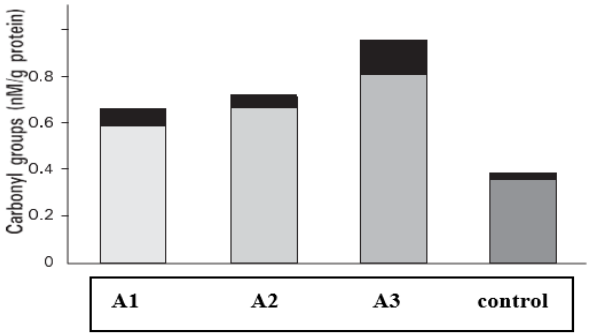

Fig. 4. Concentrations of cellular carbonyl groups of plasmatic proteins in cellular divided according to the type of Aedes aegypti and in controls. The histograms indicate mean values, and the gray areas indicate the standard error. The level of significance was P<0.01 for the difference between polyarticular subgroup and controls, and P<0.001 for the difference in oligoarticular and systemic subgroups in comparison to controls

(**Table 3**). Average GSH concentration in the oligoarticular subgroup was slightly lower than that of controls; this difference, however, was not statistically significant. Average GSH concentration in the systemic subgroup presented lower values in relation to those of controls and other subgroups. Comparison between first group, second group, and third subgroups of samples indicated a significant difference between first group and third subgroups (P<0.02).

Enzymatic activity of GST was lower in the whole group of treatments groups with Aedes aegypti and in subgroups of the cellular samples of Aedes aegypti when compared to controls (**Fig. 4**); these differences were statistically significant (P<0.0001). Differences between subgroups of the cellular samples of Aedes aegypti were not significant (Table 3).

The enzymatic activity of cellular GST was higher in the whole group of individuals of Aedes aegypti and in subgroups of treatment when compared to controls; in this case, samples in the systemic subgroup presented

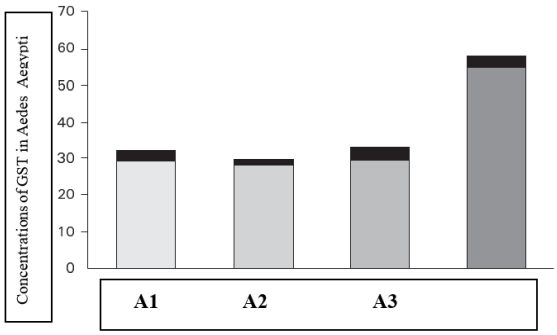

Fig. 5. Concentrations of GST in studied group divided according to the type of Aedes aegypti and in controls. The histograms indicate mean values, and the highlighted areas indicate the standard error. The level of significance of the first group, second, and third subgroups, in comparison to controls, was P<0.001

the highest values. Differences were statistically significant when comparing Aedes aegypti samples (P<0.0001), first subgroup (P<0.01), second subgroup (P<0.01), and third subgroup (P<0.0001) to the control group **Table 3** and **Fig. 5**.

DISCUSSIONS

TiO2 surface magnesium doping was likewise explored by XRD computations. Both the rutile (110) and anatase (101) surfaces were considered [295, 296]. Closely resembling substitutional and interstitial species were distinguished as in the main part of TiO2. The electronic exchange with oxygen opening is observed to be synergistic; affirming that surface Mg-doping is relied upon to cause an upgraded imperfection fixation. Pillai and co laborers (Macoris et al., 2007), watched a startling blue move in the UV/Vis absorbance of Mg–TiO2 tests warmth treated at ≥500 ◦C. XRD examination demonstrated that these Mg doped TiO2, handled huge measures of anatase stage. Di Valentin and collaborators [297] had recently clarified utilizing hypothetical figuring's that a blue move was seen with Visible-Light Activation of TiO2 Photocatalysts the theoretical insights can successfully be employed to develop novel catalysts to enhance the photocatalytic performance in the visible region. Recent developments in the theory and experiments in visible-light induced water splitting, Degradation of environmental pollutants, water and air purification and antibacterial applications are also reviewed. Various strategies to identify appropriate dopants for improved visible-light absorption and electron-hole separation to enhance the photocatalytic activity are discussed in detail, and a number of recommendations are also presented. Experimentally, all of Aedes aegypti treated with Visible-Light Activation of TiO2 Photocatalysts by nitrogen with three concentrations, after that we were collected all samples to calculated the concentrations of MDA .

Table 4. Markers of antioxidant defense system activity in Aedes aegypti, in experimental subgroups, and in controls

Row	Treatment groups (n=64)	First group (n=17)	Second group (n=35)	Third group (n=12)	Controls (n=60)
SOD	9.22±0.2	8.8±0.33	9.34±0.3	9.47±0.4	7.84±0.21
(U/ml)	P<0.0001	P<0.05	P<0.0001	P<0.001	
GSH	1207.94±75.3	1106.5±153.6	1353.7±104.8	926.5±106.23	1394.0±47.04
(mM)	P<0.05	P<0.05	NS	P<0.01	
GST	28.46±1.51	28.92±3.12	27.96±1.96	29.30±3.8	54.49±3.25
(U/g)	P<0.0001	P<0.0001	P<0.0001	P<0.0001	
GSH-Red	94.41±3.85	91.59±7.22	87.82±3.6	117.64±12.97	75.63±1.3
(U/L)	P<0.0001	P<0.01	P<0.01	P<0.0001	

SOD: superoxide dismutase; GSH: glutathione; GSH-Px: glutathione peroxidase; GSH-Red: glutathione reductase; NS: nonsignificant, significance (P) in relation to control group

Our results aimed at determining the products of lipid and protein peroxidation in organic fluids or cells. The MDA is a product generated during enzyme oxygenation of arachidonic acid, and it is a product of the oxidative degradation of lipids (Beauchamp et al., 1971) and (Bradford, 1976) Aedes aegypti samples presented concentrations of cellular MDA significantly higher than those of controls. Our results are similar to those found by (Han-Dong & Qi-Zhi, 2012) in which cellular MDA levels were higher among samples with Aedes aegypti; this suggests that lipid peroxidation is increased among samples with inflammatory nanoparticles. Similar results were described in the mosquito's fluid. Identification of products of lipid peroxidation in the synovial fluid and in the cellular samples of Aedes aegypti has been recognized as an indirect evidence of the effect of free radicals on the pathogenesis of this case (Felton & Summers, 1995).

By dividing samples with cellular MDA according to type of Aedes aegypti, it was possible to verify significant differences in comparing the three subgroups to controls. We observed that the subgroup of samples with cellular Aedes aegypti, which is considered the most severe type of Aedes aegypti, presented higher levels of MDA. In this group, we also observed the highest recurrence of acute episodes and of samples who had been submitted to short- term treatment, which could explain the increased oxidative stress. Our observations are in agreement with results found by (Jirakanjanakit et al., 2007) and (Macoris et al., 1995) and (Jain S. & Levine, 1995) and (Mracek & Webster, 1993) which described an increase in the MDA concentration in adult samples with Aedes aegypti.

In our group of sanples, concentrations of samples LPO, which are also a product of the lipid peroxidation process, were higher in comparison to controls. This difference, however, was not statistically significant. Yet, by dividing (Mracek & Webster, 1993) samples into subgroups according to type of parameters, it was possible to demonstrate a significant difference when comparing concentrations of plasma LPO in the cellular samples of Aedes aegypti to MDA levels the cellular samples of Aedes aegypti and to controls. Findings of higher LPO concentration among the cellular samples of Aedes aegypti are consistent with those of higher MDA concentration among these same cases (Macoris et al., 2007) and and (Jain S. & Levine, 1995).

The reaction of oxygen with unsaturated lipids can generate numerous compounds, such as HPX, which are the first products of the reaction (Macoris et al., 2007), According to (Zhang et al., 2019), high HPX concentrations in plasma are a direct evidence of the presence of oxidative stress (Wang et al., 2001).

In the whole group of samples with nanoparticles exposure, physiological levels of HPX were higher in comparison to controls, but differences were not statistically significant. The subgroup of third group presented a significant increase in HPX concentration in comparison to other subgroups of samples and to controls. This may be explained by the severity that is peculiar to cellular sample of Aedes aegypti- this subgroup presents the most important increases in lipid peroxidation.

The oxidation of proteins is typically characterized by the introduction of carbonyl groups into side-chain proteins. The quantification of protein-bound carbonyl groups allows us to carry out assays aiming at assessing oxidatively modified proteins. Several studies have described increase in the levels of carbonyl groups related to ischemia- reperfusion phenomena, correction of hyponatremia, cataract, physiological fluid samples with Aedes aegypti, aging, and cells exposed to active immune system stimuli by GST and GSH activity (Maxwell et al., 1994) and (Simon et al., 1974).

Long-term exposure of proteins to free radicals may lead to spontaneous modifications. These complex reactions result in accumulation and degradation of damaged proteins. Oxidative modification of proteins has been described as a protein marker mechanism in phenomena of destruction and replacement of proteins. Starke-Reed & Oliver34 suggest that oxidative stress may render proteases susceptible to proteolysis. It is possible that the increased oxidation of proteins in the synovial fluid of the cellular samples of Aedes aegypti is related to the high proteolysis in the MDA levels of these parameters (Wang et al., 2001).

In relation to protein carbonyl groups, which indicate the direct effect of protein oxidation, our results presented significantly higher levels of such structures in body fluids in the whole group of samples with Aedes aegypti, and in all three subgroups, in comparison to

controls. The highest carbonyl group physiological concentrations were observed in third subgroups.

Increased carbonyl content of proteins groups may destabilize or inhibit biological activity (Rahman & Macnee, 2000) Other studies have shown that the concentration of oxidized proteins increases with age and physiological activity, (Wang et al., 2001), with exposure (laboratory animals) to oxygen at 100%, and with the reduction of enzyme biological activity, resulting in an increase in cellular lability (Toubarro et al., 2009).

The superoxide radical is the first product of molecular oxygen reduction. In accumulation to its natural toxicity, it is an important source of hydroperoxides and other reactive free radicals. The activity of superoxide dismutase (SOD), a catalyst for dismutation of superoxide radicals into H2O2 and into molecular oxygen, protects cells and tissues from superoxide radicals and other peroxides, such as lipid peroxides in vivo. These enzymes represent the first line of defense against superoxide radicals, and their production is rapidly induced lower than certain circumstances, such as exposure to oxidative stress (Zhang et al., 2019) and (Cherng Shii et al., 2019) and (Beauchamp et al., 1971).

Different authors have observed that oxidative stress induces the activity of SOD in leukocytes and erythrocytes.40 Theoretically, since there is no protein synthesis in erythrocytes, there should not be any need for induction of the enzymatic activity. However, in erythrocyte-precursor cells, induction of SOD may occur following the oxidative process. This may explain the increase in erythrocyte SOD activity observed in our study when comparing the whole group of the cellular samples of Aedes aegypti, and subgroups of samples, with controls. Since SOD represents the first line of defense in the intracellular antioxidant defense system, and since its activity may be increased in order to compensate excessive production of superoxide radicals, the increase in enzyme activity suggests an adaptive response of the cellular samples of Aedes aegypti against possible damages caused by oxygen free radicals.

The glutathione s transfers and glutathione redox enzymatic cycle represents the most important intracellular defense against toxicity induced by oxygen free radicals that's produce by nanoparticles. The cycle includes glutathione (GSH), glutathione s transfers and the enzymes glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-Red). The GSH-Px enzyme uses GSH as a substratum in reactions that catalyze reduction of H2O2, of fatty acids, and organic hydroperoxides into water and hydroxylated fatty acids (Rahman & Macnee, 2000). During the reduction of peroxides, oxidized GSH is produced. The GSH-Red enzyme reduces oxidized GSH, thus regenerating GSH. Under oxidative stress, there is an excess glutathione redox cycle, and thus an increase in the concentration of oxidized GSH. As a consequence, excess GSH is eliminated through the bile (Toubarro et al., 2009).

The significant reduction of GST activity in the whole group of the cellular samples of Aedes aegypti and in subgroups of pa the cellular samples of Aedes aegypti tents when compared to controls could be explained by the reduction of GSH found in arthritically the cellular samples of Aedes aegypti since GSH is a substratum and a cofactor of GSH-Px. Also, lower levels of GSH result in lower activity of GST, which, in turn, may increase vulnerability to oxidative stress.

In addition to reduction of available GST, GSH, the reduction of GSH-Px activity may also be caused by the process of enzyme inactivation. The enzyme itself may be inactive under conditions of intense oxidative stress, which contributes to low GST activity (Toubarro et al., 2009).

Glutathione reductase plays an important role as an intracellular antioxidant in order to maintain a high GSH/ oxidized GSH ratio, which is a fundamental condition for

Protection against oxidative damage. Enzyme activity of plasmatic GSH-Red was significantly higher in the whole group of the cellular samples of Aedes aegypti and in subgroups of individual when compared to controls. This result suggests an adaptive response in samples facing an increase in oxidative stress, and could be the consequence of a process of enzyme induction (Han-Dong & Qi-Zhi, 2012).

CONCLUSION

In conclusion, we found high concentrations of products derived from lipid peroxidation while assessing samples levels of MDA, HPX, LPO, and the oxidative damage of circulating protein according to carbonyl content of plasmatic protein. Our results suggest the association of cellular damage caused by oxygen free radicals with the pathogenesis of cellular sample of Aedes aegypti. The increased activity of the antioxidant SOD enzyme, and the modifications observed in the glutathione redox cycle, which includes GSH, and GSH-Px and GSH- Red enzymes, assessed as a whole, confirm a reduction of intracellular defense protection against toxicity induced by oxidative stress in these treated samples. third group with cellular sample of Aedes aegypti presented highly marked modifications related to the presence of systemic oxidative stress, characterized by intense lipid and protein peroxidation and reduced antioxidant defense system.

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