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EXPOSURE TO QUININE-LOADED NANOCAPSULES IMPROVE HEMATOLOGICAL, BIOCHEMICAL AND OXIDATIVE PARAMETERS IN WISTAR RATS.

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ABSTRACT
Malaria is a parasitic disease, considered a global public health problem. The most classic drug is quinine which has limited use because of their toxicity and the appearance of new drugs. In this context, the nanotechnology would be an alternative to overcome the limitations of treatment with quinine. This study aimed to verify hematological, biochemical and oxidative stress parameters in rats treated with quinine- loaded nanocapsules. The animals were divided into four groups: control (saline - 0.9% NaCl), free quinine (75mg/kg/day), blank nanocapsules and quinine-loaded nanocapsules (75 mg/kg/day). The formulations were administered three times a day intraperitoneally for seven consecutive days. On the eighth day the rats were euthanized and venous blood was collected for followed analysis. Treatment with quinine-loaded nanocapsules has shown maintain blood levels within the normal range and the markers of cardiac, hepatic and renal function decreased compared to the treated group with free quinine. In addition, the nanoencapsulation was able to significantly increase the activity of antioxidant enzymes and mitigate the lipid, protein and genetic damage induced by free quinine. Then, the results suggest that the nanoencapsulation of quinine was effective in reducing the toxicity caused by the drug, making it safer for the treatment.

KEYWORDS: quinine, nanocapsules, toxicity, hematology, biochemistry, oxidative stress.

1. INTRODUCTION
The parasitoses are infectious diseases caused by protozoan or metazoan, considered a worldwide public health problem. It is among the leading causes of death and sometimes limiting the development and the quality of life in many countries (França et al., 2008).

Among the major parasitic diseases is malaria which, according to the World Health Organization (2014), affected about 198 millions people and caused about 584 000 deaths in 2013, with 90% of these deaths occurred in Africa. In Brazil, in 2011, there were 270 000 cases, 99% of them in the Amazon region (Brasil, 2013b).

Malaria is caused by protozoa of the genus Plasmodium. In humans, the most common species are P. vivax, P. malariae, P. ovale, P. falciparum and, more recently, P. knowlesi. It is transmitted through the bite of infected female Anopheles mosquito (França et al., 2008; Hortelano et al., 2013; White, 2008).

Symptoms of malaria include fever, chills, headache, vomiting, diarrhea, acidosis and anemia, if not treated properly can lead to complications such as thrombocytopenia, renal failure, pulmonary edema, coma and death (Hortelano et al., 2013; Rolling et al., 2013). Treatment of this parasitosis aims to stop the blood reproductive cycle of parasite, eliminate the gametocytes and prevent infection relapse (Brasil, 2010). Among the various drugs available for the treatment of malaria, one of the most classic is quinine (Greenwood et al., 2008).

Quinine is an alkaloid extracted from the bark of the Cinchona tree, according to the first reports, around 1600 was used by Indians for treatment of febrile states (Achan et al., 2011; Samanidou et al., 2005). This drug acts on erythrocyte form of the parasite, the main antimalarial until the last century. However, this drug had limited use, for both therapeutic dose as for overdose is connected to a triad of toxicity involving hypoglycemia (Okitolonda et al., 1987; White, 1992), hypotension (White, 2007) and cinchonism (Achan et al., 2011; Wolf et al., 1992). Moreover, it can cause visual

(Vusirikala et al., 2005) and auditory (Gürkov et al., 2008; Tange et al., 1997) disturbances, that may be reversible. However, the World Health Organization (2015) recommends treatment with quinine + clindamycin, for seven days, in cases of falciparum malaria uncomplicated during the first trimester of pregnancy.

The mechanism of action of quinine is not fully established yet, but believed that it inhibits the formation of hemozoin and thus induces the production of pro-oxidants (Bedolla-Duran et al., 2013). The storage of pro-oxidants, also called reactive species, associated with a decrease in antioxidant defenses creates an imbalance triggering oxidative stress, which plays an important role in development of complications caused by malaria (Halliwel, 2011; Percário et al., 2012). A study in the myocardium of mice treated with quinine showed that the drug was able to induce oxidative stress generating lipid damage, evidenced by the increase in the level of malondialdehyde (Ofusori et al., 2008).

Studies suggest that nanoencapsulated systems protect the drug toxicity of the body and increase its bioavailability at the site of action, thereby decreasing their therapeutic dosage (Kumar et al., 2013; Parven et al., 2012). Haas and collaborators (2009) conducted a study with rat infected with P. berghei and found that treatment with nanocapsules containing quinine (75 mg / kg / day) resulted in 100% survival, and this dose is approximately 30% lower compared the dose effective free quinine (105 mg / kg / day). Furthermore, the nanoencapsulation allow greater interaction between the drug and the erythrocyte. However, not much known about the toxicity of nanocapsules (NC), as well as on the effects of their exposure (Elsaesser and Howard, 2012).

Thus, the objective of this study was to evaluate hematological, biochemical and oxidative stress parameters in Wistar rats treated with quinine-loaded nanocapsules.

2. MATERIAL AND METHODS

2.1. Materials and reagents

All reagents were of analytical grade, purchased from Sigma Chemical Co. (St. Louis, MO, USA). Kits for the blood profile analysis and biochemical markers of cardiac, hepatic and renal function (Labtest, Lagoa Santa, Minas Geras, Brazil).

2.2. Experimental Animals

This study was approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Pampa (UNIPAMPA) under the Protocol 033/2013, being this affiliated with the Brazilian College of Animal Experimentation (COBEA). The experiments were conducted in accordance with ethical and technical principles of animal experimentation established by the National Council for the Control of Animal Experimentation (CONCEA) and the law No. 11.794 8 October 2008 laying down the procedures for the scientific use of animals (Brazil, 2008; 2013).

We used 24 adult male Wistar rats, weighing about 150g, which remained in the Bioterio from UNIPAMPA, campus Uruguaiana under standard environmental conditions, kept in the cabin with cycle 12 hours light dark, which received diet and water ad libitum, in quantity and appropriate quality to maintain their health.

The dose of quinine (75 mg / kg / day) of the nanocapsules was established as study Haas and collaborators (2009) and it was decided to use the same dose for free quinine. Thus, animals were divided into four groups (n=6) which received the following treatments: control (saline - 0.9% NaCl), free quinine (75mg / kg / day), blank nanocapsules, quinine-loaded nanocapsules (75 mg / kg / day).

The treatment was carried out in the short term, for seven consecutive daysand the formulations were administered three times a day, always at the same time. On the eighth day the animals were euthanized, venous blood was collected and fractionated to evaluation hematological, biochemical and oxidative stress parameters.

2.3. Preparation of suspensions of nanocapsules

The NC were prepared using interfacial precipitation of preformed polymer method according to the methodology described by Fessi and collaborators (1988). The organic phase was formed with poly (ε-caprolactone), medium chain triglyceride (TCM), Lipoid S45® and quinine, and dissolved in acetone, being kept under heating and stirring. This phase was poured into the aqueous phase consisting of water and polysorbate 80. After the formation of the suspension of NC, acetone and part of the water were evaporated (2 mg / ml quinine). Blank NC, without the drug, were also prepared.

2.4. Physico-chemical characterization of nanocapsules

The formulations were characterized by diameter (Mastersizer 2000), the Zeta potential (Zetasizer, Malvern), pH and drug content.

2.5. Hematological parameters

Samples of venous blood were collected in tubes containing EDTA (Vacutainer -Becton, Dickinson and Company - New Jersey - USA) and analyzed immediately after collection. The complete blood count was performed in an automatic counter Hematology Analyzer Cell-Dyn (Abbott Diagnostics, Santa Clara, CA, USA), blood smears were prepared and stained with Panótico kit (Renylab Chemicals & Pharmaceuticals, Barbacena - Minas Gerais, Brazil).

2.6. Biochemical parameters

A part of blood collected was packaged in tubes without anticoagulant (Vacutainer - Becton, Dickinson and

Company - New Jersey - USA). Markers of cardiac function creatine kinase (CK) and isoenzyme creatine kinase cardiac muscle (CK-MB), hepatic function aspartate transaminase (AST) and alanine transaminase (ALT) and renal function urea and creatinine in serum were evaluated using automatic analyzer A25 Biosystems (AS Biosystems, Barcelona, Spain). Homocysteine levels were quantified in erythrocytes by high-performance liquid chromatography coupled to mass spectrometry (LC-MS / MS) according to the methodology developed by Nelson and collaborators (2003). All assays were performed in triplicate.

2.7. Oxidative stress parameters

The activity of the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) was determined in erythrocytes using commercial kits RANSOD® and RANSEL®, respectively (Randox Laboratories, UK). The activity of catalase (CAT) in erythrocytes was determined according to the method described by Aebi (1984). Total glutathione (GSH) levels in erythrocytes were quantified through the method described by Akkerboom and Sies (1981).

The oxidative damage to plasma proteins was determined by measurement of carbonyl group second method of Levine and collaborators (1990). Lipid peroxidation was determined in plasma by measuring the thiobarbituric acid reactive species (TBARS) according to the methodology described by Ohkawa and collaborators (1979). The frequency of micronuclei was determined in peripheral blood leukocytes second the methodology described by Schmid (1975). All assays were performed in triplicate.

2.8. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). The comparison among groups was performed using one-way analysis of variance (ANOVA) followed by post hoc Bonferroni test for multiple comparisons. The results were considered statistically significant when $p < 0.05$.

3. RESULTS

The physico-chemical characteristics of the NC are described in Table 1. The nanoparticles, generally, have an average diameter between 100 and 300 nm and its size may vary owing to different factors, such as preparation method and, in the case of the NC, the nature of oil used in the core (Schaffazick et al., 2003). In this work the NC had an average diameter of 160.9 ± 2.2 nm to 176 ± 8 nm, showing that they are considered NC. The zeta potential, in turn, indicates the electric potential of the surface of the nanoparticles, being influenced by the load of formulation components (Schaffazick et al., 2003). Blank nanocapsules, in this study, showed average zeta potential of -13 mV and quinine-loaded nanocapsules of -18 mV. The average pH of blank nanocapsules was 4.8 and of quinine-loaded nanocapsules was 8.4, which is higher owing to presence of drug. The drug content in the NC indicates the amount of drug that is nanocoated with relation to what has been placed for preparation of NC (Schaffazick et al., 2003). In this work, the quinine-loaded nanocapsules showed drug content of 98.6 ± 2.89%, indicating good encapsulation efficiency.

Table 1: Physico-chemical characterization of nanocapsules

	Blank Nanocapsules	**Quinine-loaded Nanocapsules**
Diameter (nm) Zeta Potential (mV)	160.9 ± 2.2 -13.4 ± 0.8	176 ± 8 -18.0 ± 2.2
pH	4.8 ± 0.1	8.4 ± 0.02
Content (%)	---	98.6 ± 2.89

The results obtained in this study for hematological parameters are shown in the Table 2. The findings show that the treated group with free quinine obtained values significantly decreased ($p<0.05$) hemoglobin (Hb), hematocrit (Hc) and total erythrocyte count (RBC) compared to the control group. The treated groups with NC had significantly higher values ($p < 0.05$) for Hb, Hc and RBC compared to free quinine group.

Table 2: Hematological parameters in Wistar rats treated with nanocapsules containing quinine.

	Control	Free Quinine	Blank Nanocapsules	Quinine-loaded Nanocapsules
Hemoglobin (g/dL)	14.02 ±0.48	11.58±0.55a	14.24±0.43b	14.23±0.27b
Hematocrit (%)	39.50±0.94	35.10±0.77a	38.93±0.57b	39.29±0.68b
Red blood cells (RBC) (106/mm³)	6.52±0.45	5.36±0,17a	6. 41±0.17b	6.42±0.23b
MCV (fL)	57.83±1.09	50.67±1.53a	57.00±0.77b	57.56±0.51b
MCH (pg)	20.47±0.467	19.38±0.77a	20.51±0.78b	20.30±0.86b
MCHC (%)	35.28±0.83	32.03±0.49a	34.28±0.91ab	34.99±0.95b
RDW	14.80±0.43	15.15±0.24a	14.93±0.37	14.98±0.40
Leukocytes (103/ mm³)	6.10±0.24	4.68±0.45a	6.27±0.20b	6.30±0.28b
Neutrophils (%)	33.00±7.05	33.17±4.73	32.44±7.20	38.80±11.08
Lymphocytes (%)	61.67±7.02	60.33±3.79	61.94±7.09	57.60±9.22
Eosinophils (%)	1.56±0.92	1.72±0.46	2.16±1.15	2.00±0.00
Monocytes (%)	3.67±1.857	4.83±1.25	3.67±1.49	3.89±1.94
Band neutrophil (%)	1.00±0.00	1.00±0.00	1.00±0.00	2.27±1.56abc
Platelets (10³/mm³)	324.33±19.99	96.33±5.87a	952.61±49.75ab	722.63±63.91abc

[a]Significantly different of the control group ($p < 0.05$); [b]Significantly different of free quinine group ($p < 0.05$); [c]Significantly different of blank nanocapsules group ($p < 0.05$).

The total number of leukocytes was significantly decreased ($p < 0.05$) in the free quinine group compared to the control group and significantly increased ($p < 0.05$) in the treated groups with NC when compared to free quinine group. The differential count white blood cell (WBC) had change only in the number of band neutrophil, which was significantly higher ($p < 0.05$) in the treated group with quinine-loaded nanocapsules when compared to other groups.

The platelet count, in turn, was significantly decreased ($p < 0.05$) in the free quinine group compared to the control group and increased in the treated groups with NC in relation to control groups and free quinine.

The findings of biochemical parameters are shown in Fig. 1. The levels of creatinine (Fig. 1A) were significantly increased ($p < 0.05$) in the free quinine group and significantly decreased ($p < 0.05$) in the treated group with blank nanocapsules compared to the control group. The treated groups with NC showed a significant decrease ($p < 0.05$) in creatinine levels of relative to free quinine group. Urea (Fig. 1B) showed a significant increase ($p < 0.05$) in the free quinine group compared to the control group and a significant decrease ($p < 0.05$) in the treated groups with NC compared to free quinine group.

In Fig. 1C and 1D are shown the results for AST and ALT, respectively. The levels of AST showed a significant increase ($p < 0.05$) in the free quinine group compared to the control group and significant decrease ($p < 0.05$) in the treated groups with NC when compared to free quinine group. The results of ALT in the free quinine group and treated group with quinine-loaded nanocapsules were statistically increased ($p < 0.05$) compared to the control group. The treated groups with NC showed significantly lower values ($p < 0.05$) when compared to free quinine group.

The results CK, CK-MB and homocysteine are shown in Fig. 1E, 1F and 1G respectively. Results for CK and CK-MB showed that there was a significant increase ($p < 0.05$) in the free quinine group compared to control group, and showed that the levels of these parameters were significantly decreased ($p < 0.05$) in treated groups with NC when compared to free quinine group. Homocysteine levels in the treated group with blank nanocapsules were statistically decreased ($p < 0.05$) compared to the control group and the free quinine group.

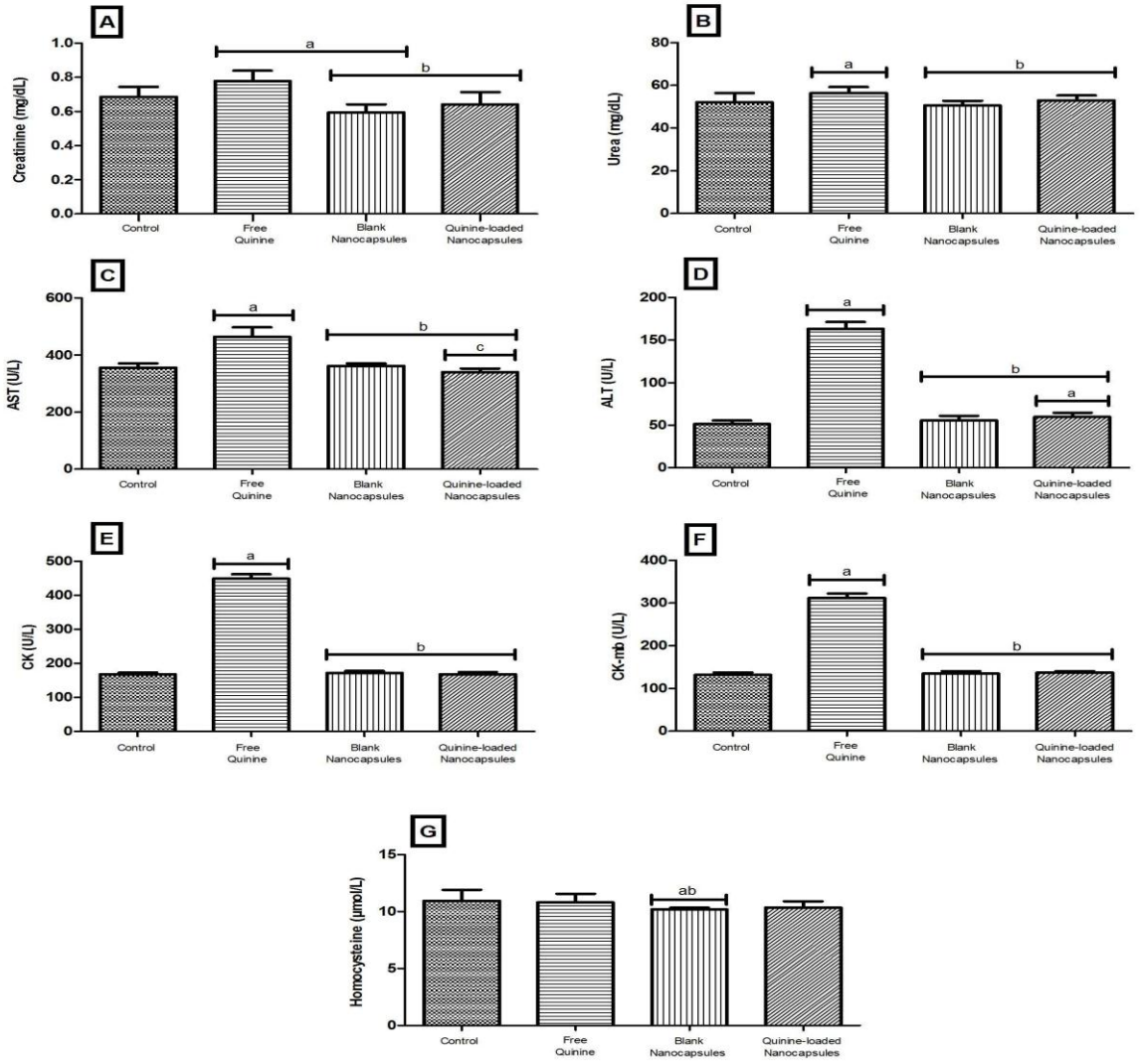

Figure 1. Biochemical parameters in Wistar rats treated with nanocapsules containing quinine. (A) Values for creatinine. (B) Values for urea. (C) Values for AST. (D) Values for ALT. (E) Values for CK. (F) Values for CK-MB. (G) Values for homocysteine.

[a]Significantly different of the control group ($p < 0.05$); [b]Significantly different of free quinine group ($p < 0.05$); [c] Significantly different of blank nanocapsules. group ($p < 0.05$).

The results obtained for the antioxidant defenses are shown in Fig. 2. The catalase enzyme activity (Fig. 2A) showed a significant reduction ($p < 0.05$) in the free quinine group compared to control group and a significant increase ($p < 0.05$) in the treated group with quinine-loaded nanocapsules compared to the free quinine group. Both SOD and GPx activity (Fig. 2B and 2C, respectively) showed a significant reduction ($p < 0.05$) in free quinine group and quinine-loaded nanocapsules compared to the control and showed a significant increase ($p < 0.05$) in the treated groups with NC compared to the quinine group. GSH levels (Fig. 2D) had significant reduction ($p < 0.05$) in the free quinine group and quinine-loaded nanocapsules group compared to the control group; treated groups with NC, in turn, achieved a significant increase ($p < 0.05$) in GSH levels as compared to the free quinine group.

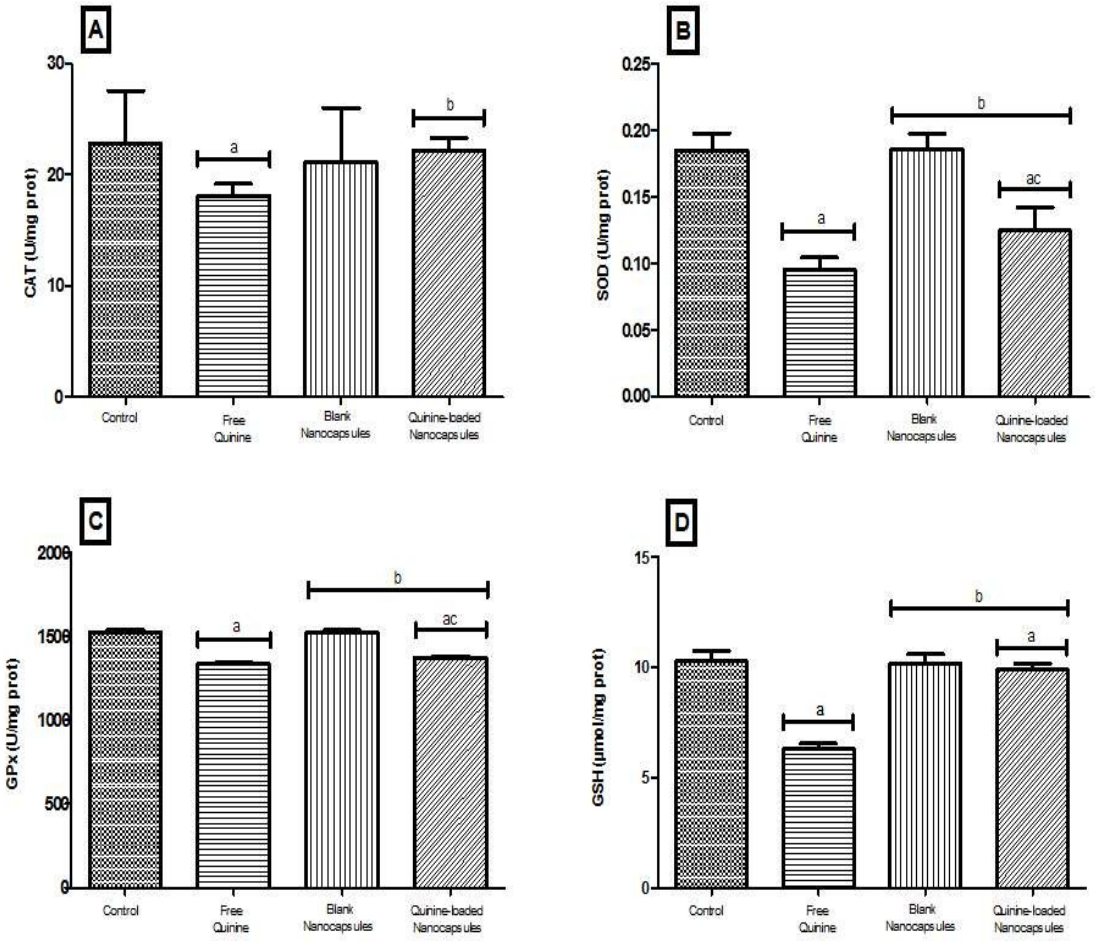

Figure 2. Antioxidant defenses in Wistar rats treated with nanocapsules containing quinine. (A) Value for CAT. (B) Value for SOD. (C) Value for GPx. (D) Value for GSH.
[a]Significantly different of the control group ($p < 0.05$); [b]Significantly different of free quinine group ($p < 0.05$); [c]Significantly different of blank nanocapsules group ($p < 0.05$).

In Fig. 3 are shown the data obtained for the evaluation of oxidative damage. The levels of TBARS (Fig.3A) demonstrated a significant increase ($p < 0.05$) in the free quinine group compared to the control group and a significant reduction ($p < 0.05$) in the treated groups with NC when compared to free quinine group. Free quinine group also showed a significant increase ($p < 0.05$) of carbonyl groups (Fig. 3B) compared to the control group; and the quinine-loaded nanocapsules group showed a significant reduction ($p < 0.05$) in this parameter compared to free quinine group. The frequency of micronuclei (Fig. 3C) demonstrated a significant difference ($p < 0.05$) of all treated groups compared to the control group. The treated groups with NC showed a significant reduction ($p < 0.05$) relative to free quinine group.

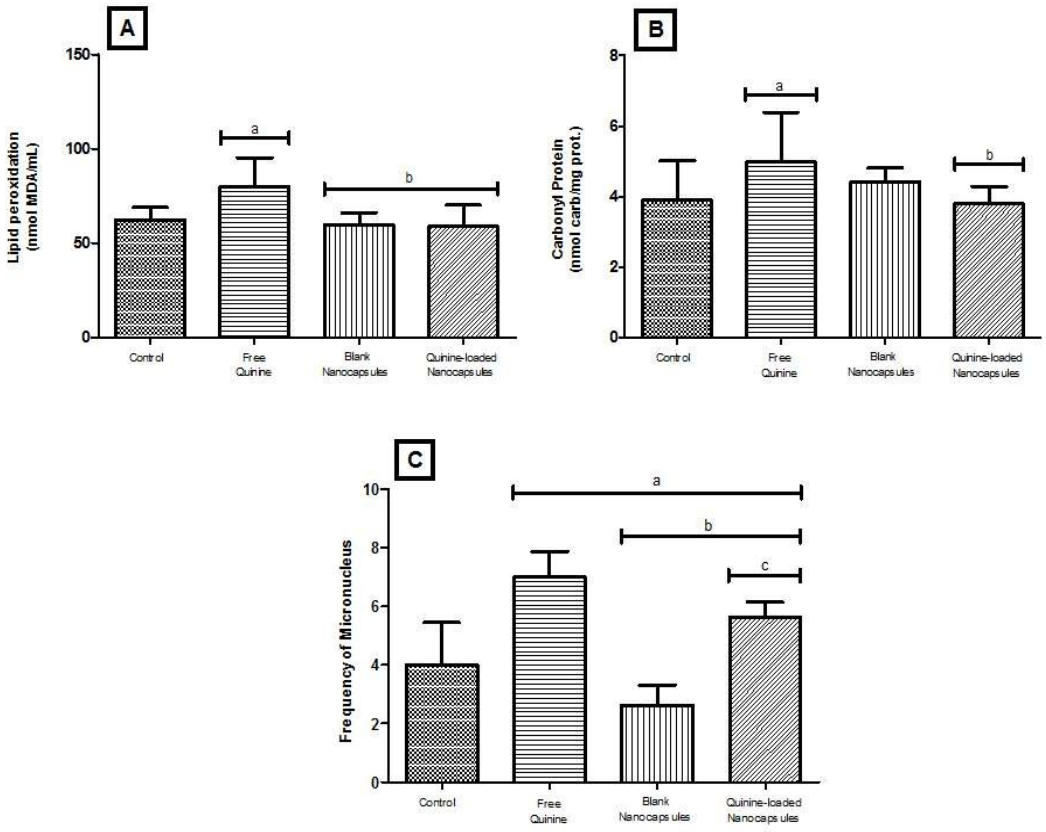

Figure 3. Markers of oxidative damage in Wistar rats treated with nanocapsules containing quinine. (A) Lipid peroxidation. (B) Protein carbonyl content. (C) Micronucleus Test.
[a]Significantly different of the control group ($p < 0.05$); [b]Significantly different of free quinine group ($p < 0.05$); [c]Significantly different of blank nanocapsules group ($p < 0.05$).

4. DISCUSSION

Malaria is an important public health problem that still causes hundreds of deaths worldwide. There are several medications available to treat this disease, which the quinine. This drug is a potent antimalarial but had limited use because of their side effects. The use of NC may be a promising alternative to protect the drug and also minimize the adverse effects caused by quinine.

The results found in the blood test showed that treatment with the free quinine induced a significant decrease in the levels of Hb, Hc and RBC, characterizing an anemia, corroborating studies conducted in patients treated with quinine, which also showed this picture (Anjelo et al., 2014; Stroncek et al., 1992). Another study by Osonuga and collaborators (2012a) showed that mice treated with artemether also had anemia. Omotosho and collaborators (2014) demonstrated that the use of chloroquine and artesunate induces a reduction in RBC and Hc, whereas the Hb levels decrease only with the use of chloroquine. According Dhaliwal et al (2004), quinine causes the production of IgM antibody, forming an antibody-drug complex which binds to erythrocytes and activates the complement system, which causes hemolysis. Regarding the white series, it was found that the treatment with free quinine changed WBC characterizing a leukopenia. These results are in agreement with studies in patients treated with quinine in which it was found that the presence of these hematological disturb (Stroncek et al., 1992; Sutherland et al., 1977). Other studies, involving different antimalarial, also demonstrated the induction capacity of WBC decrease by these treatments (Omotosho et al., 2014; Osonuga et al., 2012a; Shuhua et al., 2002).

The group receiving free quinine showed a significant reduction in the number of platelets. This finding corroborates with studies that revealed reduction platelet counts in patients treated with quinine (Gottschall et al., 1991; Howard et al., 2003). Research suggests that this drug-induced thrombocytopenia is due to an antibody, the presence of the drug, it binds to epitopes on the platelet membrane (Mintezer et al., 3662009).

The evaluation of hemogram for the treated group with quinine-loaded nanocapsules suggests that NC is able to generate less damage or normalize levels of hematological parameters compared to treatment with free quinine. Quinine is a drug that is distributed in various organs of the body. In the heart is able to block sodium channels (Jaeger, 2012), prolong the QT interval on the electrocardiogram (Kinoshita et al., 2010) and has

vasodilating action (Riseman et al., 1954). Cardiac function can be evaluated through blood biomarkers such as CK, present in the skeletal muscle may be elevated in various myopathies and CK-MB, one of the isoforms of CK, present in myocardium and considered a marker of heart damage (Nigam, 2007).

In the present study CK and CK-MB levels they found significantly increased in the free quinine group compared to the control. The same effect was observed in patients treated with chloroquine (Reffelmann et al., 2006). The cardiovascular damage and the myopathy are among the toxic effects caused by antimalarials (Alkadi, 2007), as well as cardiomyopathy (Teixeira et al., 2002). Treatment with quinine-loaded nanocapsules revealed a significant improvement of these biomarkers, suggesting a protective character of cardiac function.

The liver is also the target of quinine metabolites. The assessment of hepatic function may be performed by serum biomarkers as bilirubin, alkaline phosphatase, ALT and AST. Transaminases are recommended for analysis of hepatocellular injury and ALT is more sensitive and specific, being found principally in the liver (Singh et al., 2011). Quinine is associated with liver damage, such as granulomatous hepatitis (Katz et al., 1983; Mathur et al., 1990), but there is little evidence of their relationship to changes in transaminases (Livertox, 2015). In this study it was found a significant increase in serum levels of AST and ALT in free quinine group, suggesting hepatics implications. These findings are related to studies in patients treated with this drug (Howard et al. 2003; Katz et al., 1983; Perez et al., 1994). Another study also found that the AST and ALT average of malaria patients treated with quinine or artesunate showed up increased (Rolling et al., 2013).

Serum ALT levels showed up elevated in rats treated with quinine and rich drink quinine (Theophilus et al., 2012) as well as in children diagnosed with cerebral malaria treated with this drug (Abdalla and Zaki, 2009). Normally, quinine has mild hepatotoxic effect and the levels of biomarkers normalize after suspension of treatment, and were not related deaths due to this cause (Livertox, 2015). In addition, other antimalarials change caused these liver enzymes, such as artesunate (Bigonyaa et al., 2015), Coartem (artemether + lumefantrine) (Theophilus et al., 2012), Fansidar® (sulfadoxine + pyrimethamine) (Johnkennedy et al., 2010) and halofantrine (Osonuga et al., 2012b). In this study, the level of transaminases obtained a significant improvement after treatment with quinine nanocoated, suggesting protective character of liver function.

The toxic effects of quinine may also compromise the kidney (Achan et al., 2011; Colley et al., 1989). Renal function can be checked by changing biomarkers such as urea and creatinine (Mounton and Holder, 2006; Theophilus et al., 2012). These survey results showed an increase of these renal parameters in the treated group with free quinine. Studies in animal models and humans have shown that treatment with quinine caused an increase in creatinine level (Anjelo et al., 2014; Pawar et al., 1994; Theophilus et al., 2012) e de ureia (Anjelo et al., 2014; Colley et al., 1989). Both parameters can also be increased due to the use of drinks containing quinine (Theophilus et al., 2012). The treatment with other antimalarials, as Coartem® (Theophilus et al., 2012), Fansidar® (Jhonkennedy et al., 2010) e P-Alaxin® (Olayinka e Ore, 2013), they can also raise the level of these renal biomarke. An increase in creatinine may signal renal toxicity and elimination deficiency of residue in the body (Theophilus et al., 2012). According to this study, treatment with quinine-loaded nanocapsules presented creatinine and urea values similar to the control group.

This work also demonstrated that treatment with free quinine significantly decreased the activity of antioxidant enzymes SOD, CAT and GPx and the level of GSH. The study Olayemi and collaborators (2012) showed a decrease in SOD and glutathione S-transferase (GST) activity due to treatment with quinine and with other antimalarials. In addition, treatment with different antimalarials caused a decrease of antioxidant defenses, such as GSH levels and of GST activity in liver (Farombi et al., 2000), SOD activity in liver and CAT in liver and kidney (Adaramoye et al., 2008), SOD and CAT activity in plasma (Iyawe and Onigbinde, 2009) and SOD activity in serum (Idowu et al., 2015). Akanbi and collaborators (2010) demonstrated reduced levels of GSH and ascorbic acid in infected women or healthy women treated with different antimalarials.

Moreover, these results also showed that treatment with free quinine caused oxidative damage to biomolecules. The proteins are immediate target of oxidative stress, when oxidation occurs their side chains are produced of carbonyl groups that also may be introduced in proteins due to other reactions and can be used as biomarker protein damage, and its accumulation already has been observed in several pathologies (Dalle-Donne et al., 2003; Heidari et al., 2014). In this study it was found that the treatment with free quinine induced protein damage. This finding corroborates with studies report that exposure of human erythrocytes at primaquine (Alanazi, 2010) and hepatocytes at amodiaquine (Heidari et al., 2014) may lead to increased levels of carbonyl groups.

The lipids, in particula polyunsaturated fatty acids, phospholipid components forming the cell membrane, are vulnerable to oxidative stress. The attack of free radicals can cause lipid peroxidation and, hence, change the structure and functions of the cell membrane (Niki, 2014; Rao P et al., 2011). Treatment with free quinine in this work was able to induce lipid peroxidation. This result is in agreement with the study of Ofusori and collaborators (2008), which showed that the quinine can cause lipid damage in the heart muscle, evidenced by the

increase in TBARS levels. Other antimalarials drugs can cause lipid damage in rat liver (Farombi et al., 2000; Srivastava et al., 1993) and serum of healthy or malarial individuals (Akanbi et al., 2010), mice (Iyawe and Onigbinde 2009) and rabbits (Adisa et al., 2011). This present study also found that the treated group with free quinine showed a significant increase in micronuclei frequency. The micronucleus test is used to investigate permanent damage to the genetic material, whereas the micronucleus are fragments of chromosomes or entire portions that are not included in the nucleus during the cell division and are generated due to DNA damage (Luzhna et al., 2013). These results are consistent with studies which report that treatment with antimalarials drugs such as artesunate (Aquino et al., 2011) and Coartem® (Idowu et al., 2015) induce to an increase in micronuclei frequency in rodent bone marrow. It was also observed that the group treated with quinine-loaded nanocapsules showed a significant increase in micronuclei frequency, indicating that the NC minimize genetic damage induced by free quinine, but do not protect fully compared to the control group.

Through analyzing the antioxidant defenses and oxidative damage markers it was found that the NC were able to alleviate the oxidative stress induced by treatment with free quinine, thus minimizing the cytotoxic potential of the drug.

Nanotechnology is a therapeutic novelty, thus don't known exactly the effects of exposure to NC and its toxicity. Furthermore, the mode of interaction between the NC with the organism is not totally clarified, but scientific studies report that the cells can absorb them. It is known that there are several possible mechanisms NC cause toxicological damage, including the generation of reactive species (Elsaesser and Howard, 2012). In this study it was found that the NC was not able to induce notable toxicity in most of the parameters analyzed, in the conditions used, corroborating study of Bulcão and collaborators (2014), an important data for the evaluation of security nanoencapsulated systems.

5. CONCLUSION

The findings this work suggests that treatment with free quinine may alter hematological, biochemical and oxidative stress parameters and that the administration of nanoencapsulad drug can mitigate these damages. Thus the NC can be an effective instrument for reducing the toxicity of quinine and increase the safety of the treatment for malaria, but more studies are required to elucidate the effects of exposure to NC in the long term.

Conflicts of interest statement

All authors report no conflict of interest.

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