NISEB Journal Vol. 19, No. 1. March, 2019 1595-6938/2019
Printed in Nigeria (2019) Society for Experimental Biology of Nigeria
http://www.ojs.klobexjournals.com/index.php/nisebj

Lipid and Antioxidant Profile of Chitosan Bound Ethylacetate Fractions of *Cocos nucifera* Husk Fiber in *Plasmodium berghei* Infected Mice

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Abstract

Malaria is a parasitic disease that occurs in tropical and subtropical regions of the world. About 500 million cases of malaria occur every year, and one million people, mostly children living in sub-Saharan Africa, die as a result. This study was conducted to determine the lipid and antioxidant status of chitosan bound ethylacetate fraction of cocos nucifera husk fiber in p. berghei infected mice. Swiss albino mice were innoculated with Plasmodium berghei. The forty five mice were randomly assigned into nine groups, of 5 mice each. Administration of the Therapeutic Dose (TD) (80mg/kg) and Sub Therapeutic Dose (STD) (20mg/kg) (gotten from preliminary studies) of ethylacetate extract fraction of Cocos nucifera husk fibre coupled with chitosan and chitosan/alginate was done orally for four days post-inoculation and 0.2ml of the extract was administered. Group A served as positive control (not infected), Group B received appropriate volume of distilled water, Group C received 5mg/kg. Chloroquine (infected), Group D received 80mg/kg of extract (therapeutic dose), Group E received 20mg/kg of extract (sub-therapeutic dose, infected), Group F received 80mg/kg of extract + Chitosan + infected, Group G received 20mg/kg of extract + Chitosan + infected, Group H received 80mg/kg of extract + Chitosan/ Alginate + infected, Group I received 20mg/kg of extract + Chitosan/Alginate + infected. At the end of the experimental period, selected tissues was collected, isolated and homogenized. Antioxidant (MDA, GSH and GST) and lipid profile activities (Cholesterol, HDL and LDL) were determined. The results reveal that, there was a significantly decreased in the level of the non-treated groups as compared to other treated groups and control in cholesterol, HDL and LDL level while there was significant increase in triacylglyeride level in non – treated groups compared to other test groups and control after the administered ethylacetate fraction of cocos nucifera bound to chitosan microparticles. In present study reduced glutathione (GSH), GST and lipid peroxidation product malondialdehyde (MDA) were increased significantly compared to test groups and control. These results suggest that ethylacetate fraction of Cocos nucifera husk fibre bound to chitosan microparticles may boost body's antioxidant systems, which neutralizes the effects of free radicals and also able to reverse the change in serum lipid profile caused by malaria infection.

Keywords: Chitosan, Antioxidant, Lipid, Ethylacetate and *plasmodium berghei*

Introduction

Malaria is a parasitic disease transmitted by the bites of *Anopheles* mosquitoes infected with *Plasmodium* species, four of which infect humans: *Plasmodium falciparum* (the most deadly one), *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. The disease primarily affects poor populations in tropical and subtropical areas, where the temperature and rainfall are suitable for the development of vectors and parasites (1). More than 40% of the world population is at risk of the disease (2). An estimated 1.2 billion are at high risk of transmission (≥1 case per 1000 population), half of which live in the African regions; 80% of such cases are concentrated in 13 countries, and over half in Nigeria, Congo, Ethiopia, Tanzania and Kenya (3). Nigeria accounts for a quarter of all malaria cases in Africa (3). In the southern part of the country, transmission occurs all year round while in the north it is more seasonal. Almost all malaria cases in the country are caused by *Plasmodium falciparum*, considered to be the leading cause of death worldwide in 2004, from a single infectious agent (4). Malaria is the most common disease in Nigeria; according to the (5), half of its population will have one or more malaria attacks annually. The continuous spread of *Plasmodium falciparum* resistance to antimalarial drugs poses a serious threat to malaria control programs. In Nigeria, a nationwide surveillance data on drug efficacy showed that chloroquine (CQ) and sulphadoxine-pyrimethamine (SP) are no longer viable therapeutic options for the effective treatment of human malaria (5, 6).This, in addition to the increased number of drug-resistant parasites, makes the

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development of novel antimalarial urgent. The high cost of malaria treatment has left the poor masses of Nigeria heavily reliant on traditional practitioners and medicinal plants for the treatment of the disease.

Traditional herbal medicines have been used to treat malaria for thousands of years in various parts of the world. The first antimalarial drug used in the Occident was extracted from the bark of the *Cinchona* (Rubiaceae) species, the alkaloid quinine, still largely used. Infusions of the plant bark were used to treat human malaria as early as 1632 (6). Years later quinine was isolated and characterized (7), thus becoming the oldest and most important antimalarial drug. Another ancient medicinal plant of millennium use in the West is *Artemisia annua*, rediscovered in China in the seventies as an important source of the antimalarial artemisinin (8; 9; 10). Artemisinin-combined therapies (ACT) were formally adopted as first-line treatment of uncomplicated malaria in Nigeria from 2005 onwards (11). However, ACT use is limited due to its high costs, limited production of artemisinin derivatives to Good Manufacturing Practices (GMP) standards and toxicity (12, 13, 14, 17, 15, 16, 18, 19).

With the problems of increasing levels of drug resistance and difficulties in poor areas of being able to afford and access effective antimalarial drugs, traditional medicines could be an important and sustainable source of treatment Thus, the use of plant remedies has steadily increased worldwide in recent years, as well as the search for new phytochemicals that could be developed as useful drugs for the treatment of malaria and other infectious diseases. In Nigeria, the coconut palm is found mostly in the Southern states and in some marginal areas up to 10^{o}N. The largest coconut palm plantation is found in the Badagry local government area of Lagos State located in the South West of Nigeria.

Cocos nucifera husk fibre and white flesh are used in folk medicine for the treatment of malaria. Despite the fact that we live in an era of advanced technology and innovation, infectious diseases, like malaria, continue to be one of the greatest health challenges worldwide. The main drawbacks of conventional malaria chemotherapy are the development of multiple drug resistance and the non-specific targeting to intracellular parasites, resulting in high dose requirements and subsequent intolerable toxicity.

Nanoparticles are particulate dispersions or solid colloidal structures ranging from 1 – 1000 nm in diameter (20). They are composed of synthetic, semi-synthetic and natural polymers in which the active therapeutic molecule has the capability of being entrapped, encapsulated, dissolved, absorbed, or chemically attached (21, 22, 23). Due to their biodegradability, biocompatibility, and versatility in application, natural hydrophilic polymers have also been extensively investigated (24). Natural polymers are classified as proteins (gelatin, albumin, lectin, legumin, and vicillin) and polysaccharides (alginate, dextran, pullulan, and chitosan). Nanosized carriers have been receiving special attention with the aim of minimizing the side effects of drug therapy, such as poor bioavailability and the selectivity of drugs. Chitosan nanoparticles offer many advantages due to their stability, low toxicity, simple and mild preparation methods, provide versatility in of administration, excellent drug deliverer, and provide optimal, controllable drug release (25).

Chitosan has gained interest in malarial treatment due to its biodegradability, biocompatibility, reduced toxicity, reduction in dose and dose frequency and carriers for controlled site-specific drug delivery (26). Chitosan bares the ability to conjugate with the age-old drug chloroquine and avoid affecting the liver and reducing mitochondrial dehydrogenase activity (27, 32). Pathology of malaria depends on the species of *Plasmodium* and strain of parasite. The use of colloidal drug carriers (liposomes and micro/nanoparticles) provide versatility in site specific or targeted drug delivery along with controlled optimal drug release (24). Nanoparticles have added advantages over microparticles such as bioavailability, the ability to improve drug encapsulation, pharmacokinetics (33).

Hence the need to formulate effective and biodegradability microparticle - based oral drug delivery system, using chitosan to overcome the side effects of drug therapy, such as poor bioavailability and the selectivity of drugs in the gastrointestinal tract.

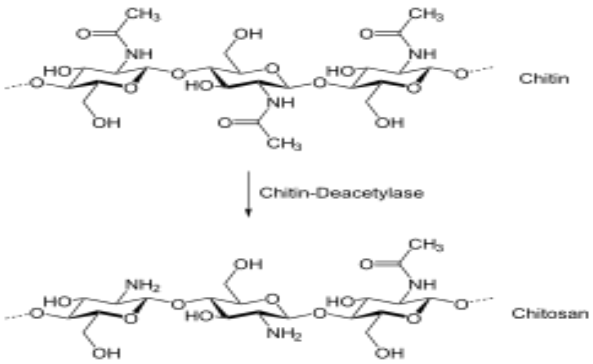

Figure 1: General Structure of Chitin and Chitosan (34)

The overall objective was to assess the lipid and antioxidant profile of chitosan bound ethylacetate fractions of *cocos nucifera* husk fiber in *plasmodium berghei* infected mice,

Materials and Methods

Plant Material

The plant material used for this research was husk fibres of *Cocos. nucifera* (West African tall variety) dried in the shade at room temperature was obtained from Nigeria Institute for Oil Palm Research (NIFOR), Badagry, Lagos State, Nigeria.

Preparation of Extracts

The extract was prepared as described in a previous study (16). The dried husk fibers of the *C. nucifera* (West African tall variety) were pulverized to powder. The powder (1000g) was successively extracted with 5L absolute methanol and 2.5L ethyl acetate for 72hours per solvent. The extracts were filtered using Whatman filter paper No. 1 and then concentrated on water bath. The concentrates was exposed to air and residual solvent was allowed to evaporate at room temperature to obtain the dry extract.

Preparation of Chitosan Microparticles

Chitosan nanoparticles was prepared by the ionic gelation of chitosan solution with anionic Sodium tripolyphosphate (TPP) (23). First, 1gram of chitosan was dissolved in 1L 2% (v/v) acetic acid aqueous solution. Then, 2grams of TPP was dissolved in 1L distilled water. Subsequently, 10ml of chitosan solution was added drop wise into 20 ml of TPP solution. Chitosan colloid microparticles was formed spontaneously under mild agitation at room temperature on a magnetic stirrer. After 15-20 minutes, chitosan colloid microparticles was centrifuged at 10,000 rpm for 15 min. Then, the supernatant was discarded and the residue was rewashed three times with distilled water to remove unbound chitosan and re-dispersed in 50ml distilled water for further use.

Loading Ethylacetate Fraction of *Cocos Nucifera* Extract to Chitosan Nanoparticles

Ranging concentrations of ethylacetate fraction of *C. nucifera* was dissolved in 80 mL of ethanol (4 mg/mL). 1ml of the ethylacetate fraction was added to 9ml of 0.1% chitosan. The solution was then added drop wise into 20ml of TPP and allowed to stir on a magnetic stirrer for 30mins. Now chitosan bound ethylacetate colloid microparticles was formed spontaneously under mild agitation at room temperature on a magnetic stirrer. The colloid formed was centrifuged at 10,000rpm for 15mins and the supernatant discarded. The unbound ethylacetate fraction was removed by centrifugation at 7000 rpm for 30 min and the pellet was washed three times with distilled water (26, 27).

Preparation of Alginate Coated Chitosan Nanoparticles

Alginate microparticles were prepared by dissolving 2g sodium alginate in 1L distilled water with mild agitation at room temperature. 8ml of the alginate solution was added to 12ml of TPP and agitated mildly on a magnetic stirrer. Then, 10ml of chitosan was added in a drop wise fashion to the alginate-TPP solution and left to stir on a magnetic stirrer for 30mins. This led to the formation of colloids of chitosan-alginate microparticles. This was then centrifuged at 10,000 rpm for 15mins and the supernatant discarded. The pellet was washed thrice with distilled water, to remove unbound chitosan and alginates (22).

Experimental Animals

Forty-five (45) swisss albino mice (*Musco muslaris*) weighing 25- 30g was used for this study. The animals were obtained from the animal holding unit of University of Ilorin, Ilorin, Kwara State. They were allowed to acclimatize to the new environment for a period of two weeks prior to the study. Mice were housed in plastic cages with perforated nets at the cover to aid exchange of air (oxygen). The cages were cleaned daily, water and food changed. They were maintained under standard conditions and had access to feed and water *ad libitum*.

Innoculation of the Animals with Parasite

The mice were inoculated intraperitionally with *P. berghei* NK 65 (a chloroquine sensitive parasite strain). The tail blood was obtained from a donor mouse of known parasitemia into a sample bottle containing 2ml of 3.8% sodium citrate with 0.5% glucose solution. This was then diluted appropriately using same solution to obtain an inoculum size of 1×10^5 infected red blood cells, which was used to inoculate each mouse as described by (16).

Experimental Design

The forty-five mice was randomly assigned into nine groups, of 5 mice each. Administration of the Therapeutic dose (TD) (80mg/kg) and Sub therapeutic dose (STD) (20mg/kg) of ethylacetate extract fraction of *Cocos nucifera* husk fibre coupled with chitosan and chitosan/alginate was done orally for four days post-inoculation and 0.2ml of the extract was administered as follows:

Group A (Control): Not infected, not treated. Administered 0.2ml of distilled water solution.
Group B (Negative control): Infected, not treated. Administered 0.2ml of distilled water solution.
Group C: Infected and administered 5 mg/kg body weight of chloroquine.
Group D: Infected and administered TD of extract fraction (80mg/kg).
Group E: Infected and administered STD of extract fraction (20mg/kg).
Group F: Infected and administered TD of extract fraction coupled with Chitosan (80mg/kg).
Group G: Infected and administered STD of extract fraction coupled with Chitosan (20mg/kg).

Group H: Infected and administered TD of extract fraction coupled with Chitosan/Alginate (80mg/kg).
Group I: Infected and administered STD of extract fraction coupled with Chitosan/Alginate (20mg/kg).

Preparation of Serum and Tissue Homogenates

The animals were made unconscious using diethyl ether as an anaesthesia after which they were sacrificed via the Jugular vein puncturing method. Blood was collected both in EDTA bottles and plain sterile sample bottles for haematological analysis and biochemical assay respectively. The blood in the plain bottles were left for 30 minutes to coagulate properly after which they were spin in a Uniscope Laboratory Centrifuge at 3000rpm for 10 minutes, the sera were thereafter aspirated using Pasteur pipette into dry sterile sample bottles (35). While the blood collected in EDTA bottles were immediately taken for haematological analysis at chemical pathology department of University of Ilorin teaching hospital.

Further on, the animals were dissected quickly and the liver and kidney were removed, weighed and homogenized in ice cold 0.25M sucrose solution (1:5 w/v) to obtain the crude homogenate. The homogenates were centrifuged at 4000rpm for 10minutes and the supernatant was aspirated into small storage containers and stored in freezer at 4^0C until the time of usage.

Biochemical Analysis: Antioxidant Assays

Reduced Glutathione (GSH): The level of GSH in the liver homogenate was determined using the procedure described by Ellman (36). Briefly, 1.0 mL of liver homogenate was added to 0.1 mL of 25% trichloroacetic acid (TCA) and precipitate was removed by centrifugation at 5,000 *g* for 10 min. Supernatant (0.1 mL) was added to 2 mL of 0.6 mM DTNB prepared in 0.2M sodium phosphate buffer (pH 8.0). Absorbance was read at 412 nm.

Glutathione Transferase (GST): GST level in the serum was determined using the method of (37). 1mM CNDB was added to buffer containing 1mM GSH and an aliquot of the sample. Upon addition of CNDB the change in absorbance at 340nm was measured as a function of time.

***Malondialdehyde*: B**riefly, liver homogenate was mixed with TBA/TCA/HCl (15%, 0.2 N, 0.37%) at reagent/sample ratio of 2:1 (v/v), placed in a boiling water bath for 15 min, cooled to room temperature and centrifuged at 1000 *g* for 10 min at room temperature. The absorbance of the solution was read at 535 nm against the blank (containing all reagents except liver homogenate). MDA content was determined using the extinction coefficient of 1.56 x 10^6 (38a, 38b).

Lipid Profile

***Total Cholesterol*:** The level of cholesterol was determined using enzyme saponification procedure as described by (38, 39). The Cholesterol level was determined using appropriately diluted samples. . The reaction mixture of the standard was constituted by adding 10 µl of the standard to 1000 µl of the reagent while that of the sample was constituted by adding 10 µl of the sample to 1000 µl of the reagent. The reaction mixtures were incubated for 5 minutes at 37^0C, Absorbance was read at 546nm and the concentration of total cholesterol calculated thus:

$$\text{Concentration of Total Cholesterol in sample} = \frac{\Delta\text{A sample}}{\Delta\text{A standard}} \times \text{Concentration of Standard}$$

***Determination of Triglyceride (TRIGS)*:** The level of Triglyceride was determined calorimetrically using the method described by (40) based on the principle of enzymatic hydrolysis by lipase method by (41, 42, 43) The triglyceride level was determined using appropriately diluted samples. The reaction mixture of the standard was constituted by adding 10 **µl** of the standard to 1000 **µl** of the reagent while that of the sample was constituted by adding 10 **µl** of the sample to 1000 **µl** of the reagent. The reaction mixtures were incubated for 5 minutes at 37^0C, Absorbance was read at 565nm and the concentration of triglyceride calculated thus:

$$\text{Concentration of TRIGS in sample} = \frac{\Delta\text{A sample}}{\Delta\text{A standard}} \times \text{Concentration of Standard}$$

***Determination of HDL Cholesterol*:** The HDL level was determined using the method described by (44, 45). The HDL Cholesterol level was determined using appropriately diluted samples. The reaction mixture of the sample was constituted by adding 500 µl of the sample to 1000 µl of the precipitant. The reaction mixture was thoroughly mixed, incubated for 10 minutes at room temperature Centrifuged at 400rpm for 10 minutes. The clear supernatant was separated within 2 hours and the cholesterol content was determined.

***Cholesterol CHOD- PAP Assay*:** The Cholesterol CHOD-PAP level was determined using appropriately diluted samples. . The reaction mixture of the standard was constituted by adding 100 µl of the standard to 1000 µl of the reagent while the reaction mixture of the sample was constituted by adding 100 µl of the sample to 1000 µl of the reagent (46, 47, 48). The reaction mixture was thoroughly mixed, incubated for 5 minutes at 37^0C, the absorbance was read at 546nm and the concentration of HDL Cholesterol in sample thus:

$$\text{Concentration of HDL Cholesterol in Sample (mmol/l)} = \frac{\Delta\text{A sample}}{\Delta\text{A standard}} \times \text{Concentration of Standard}$$

***Determination of LDL Cholesterol*:** The LDL Cholesterol level was calculated using the formula as described by (49). The Friedewald equation is as follows:

$$LDL\ Cholestrol\ (mmol/l) = Tot.\text{Chol} - \text{VLDL} - \text{HDL Chol}$$

Statistical Analysis

Experimental data are presented as Means ± Standard error of mean (SEM). Statistical analysis was implemented using SPSS 20.0 version statistical package program (SPSS, Chicago, IL). One-way analysis of variance was

used to compare variables among the different groups. Level of significance (Post hoc comparisons) among the various treatments was determined by Duncan's Multiple Range Test. The differences were considered statistically significant at $*p<0.05$.

Results

Lipid Profile

***Triglycerides*:** The administration of the ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles investigated in this study did not cause any significant change ($p>0.05$) to triglyceride levels compared to the controls and other test groups

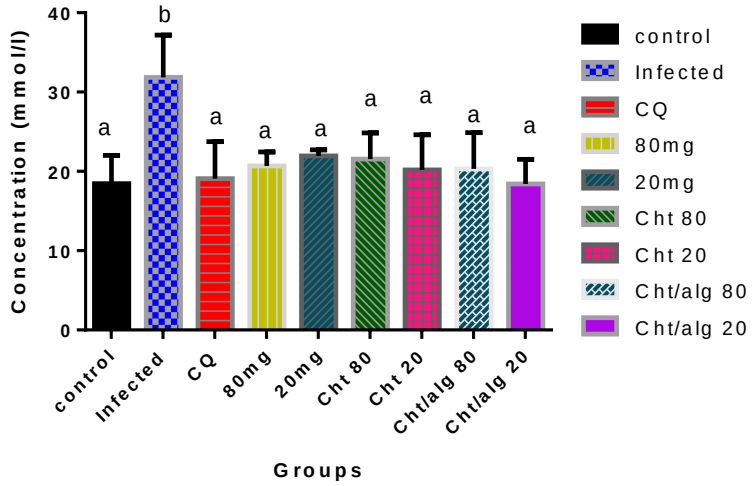

Figure 1: Triglyceride profile of mice administered ethylacetate fraction *of Cocos nucifera* bound to chitosan nanoparticles are means± SEM of 5 replicates.

***Cholesterol*:** The administration of the ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles caused significant decrease ($P<0.05$) in cholesterol level in non-treated group compared to the other test groups.

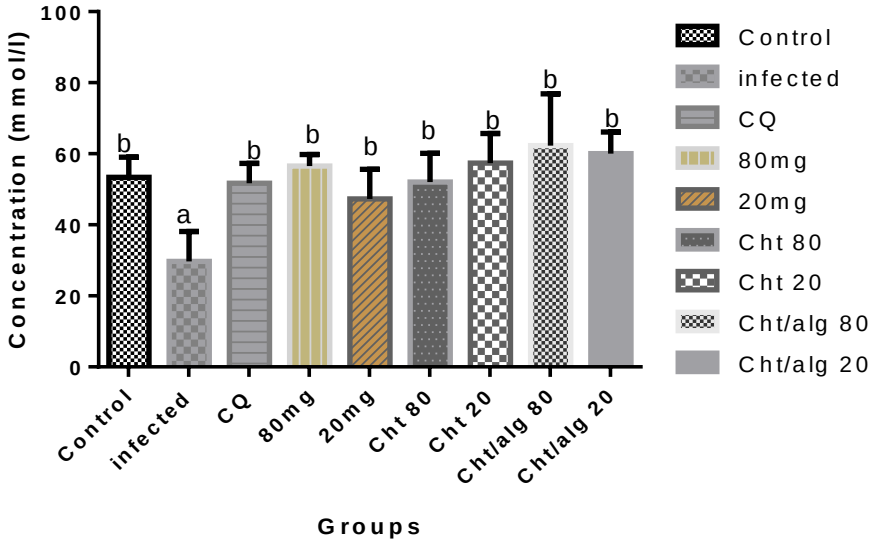

Figure 2: Cholesterol profile of mice administered ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles are means± SEM of 5 replicates.

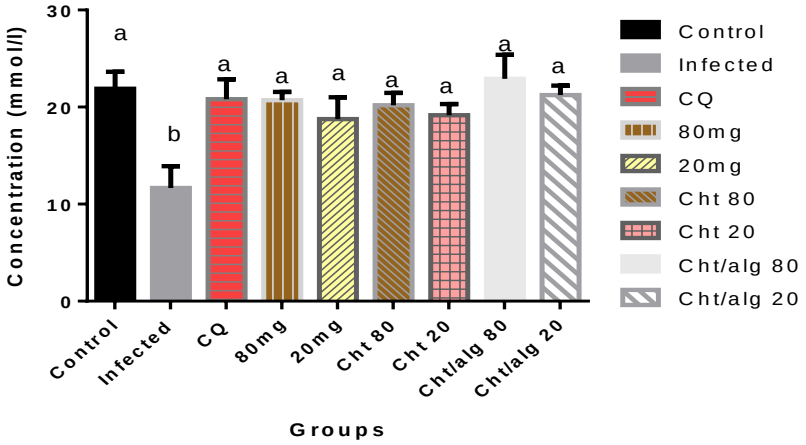

Figure 3: High Density Lipoprotein profile of mice administered ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles are means± SEM of 5 replicates.

***High Density Lipoprotein*:** The administration of the ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles investigated in this study cause significant decrease ($p>0.05$) in HDL level in non-treated groups compared to other test groups and control.

***Low Density Lipoprotein*:** The administration of the ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles caused significant decreased in non-treated groups compared to other test groups and control that are stable.

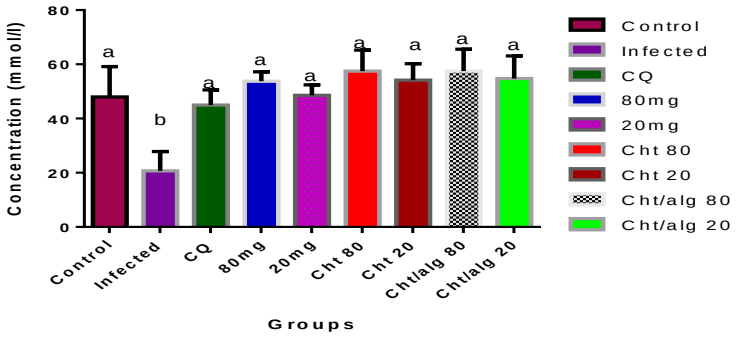

Figure 4: Low Density lipoprotein Profile of mice administered ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles are means± SEM of 5 replicates.

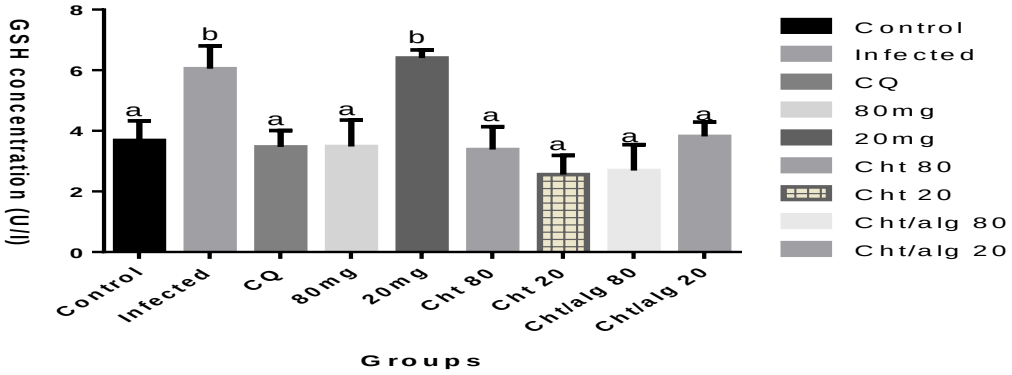

Figure 5: GSH level in kidney of mice administered ethylacetate fraction *of Cocos nucifera* bound to

chitosan nanoparticles are means± SEM of 5 replicates

Antioxidant Parameters

***GSH Kidney*:** The administration of the ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles caused significant increase in GSH levels in kidney P > 0.05 in treated groups relative to the control but decreased in infected compared to the control and other test groups that are stable.

***GSH Liver*:** The administration of the ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles caused significant decreased in GSH levels in liver P < 0.05 in treated groups relative to the control but increased in infected compared to the control and other test groups that are stable.

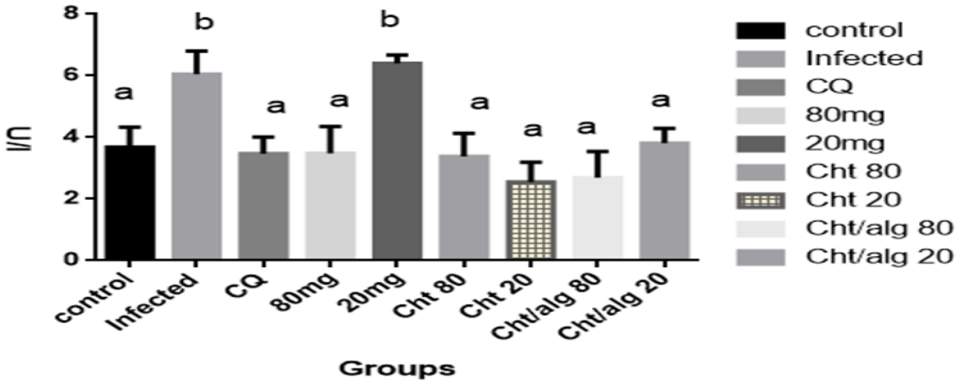

Figure 6: GSH level in liver of mice administered ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles are means± SEM of 5 replicates

***GST Kidney*:** GST level in the kidney of mice administered ethylacetate fraction of *Cocos nucifera* bound decreased significantly p < 0.05 in treated groups and relative control but increased significantly p> 0.05 in infected compared to treated groups and relative control.

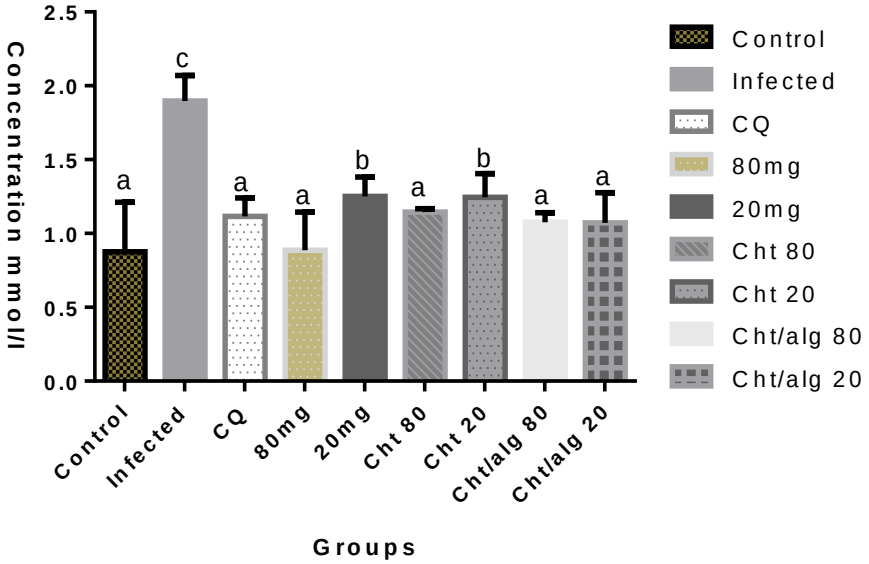

Figure 7: GST level in kidney of mice administered ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles are means± SEM of 5 replicates.

***GST Liver*:** GST level in the liver of mice administered ethylacetate fraction of *Cocos nucifera* bound increased significantly p > 0.05 in treated groups and relative control but decreased significantly p <0.05 in infected compared to treated groups and relative control.

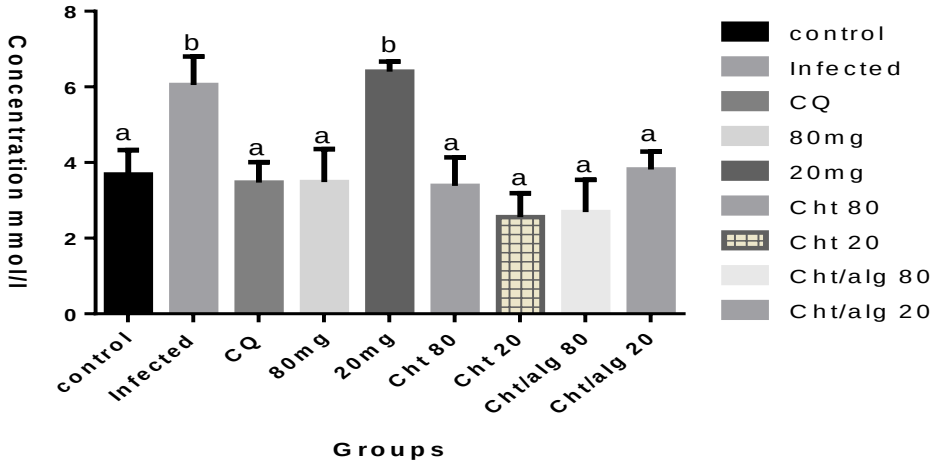

Figure 8: GST level in liver of mice administered ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles are means± SEM of 5 replicates

***MDA Kidney*:** MDA level in the kidney of mice administered ethylacetate fraction of *Cocos nucifera* bound decreased significantly $p < 0.05$ in treated groups and relative control but increased significantly $p > 0.05$ in infected compared to treated groups and relative control.

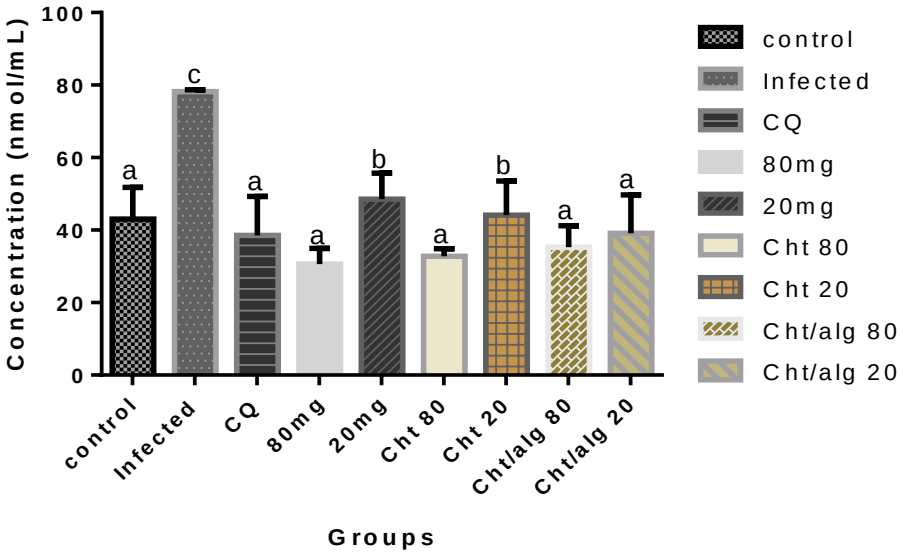

Figure 9: MDA level in kidney of mice administered ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles are means± SEM of 5 replicates

***MDA Liver*:** MDA level in the liver of mice administered ethylacetate fraction of *Cocos nucifera* bound increased significantly $p > 0.05$ in treated groups and relative control but decreased significantly $p < 0.05$ in infected compared to treated groups and relative control

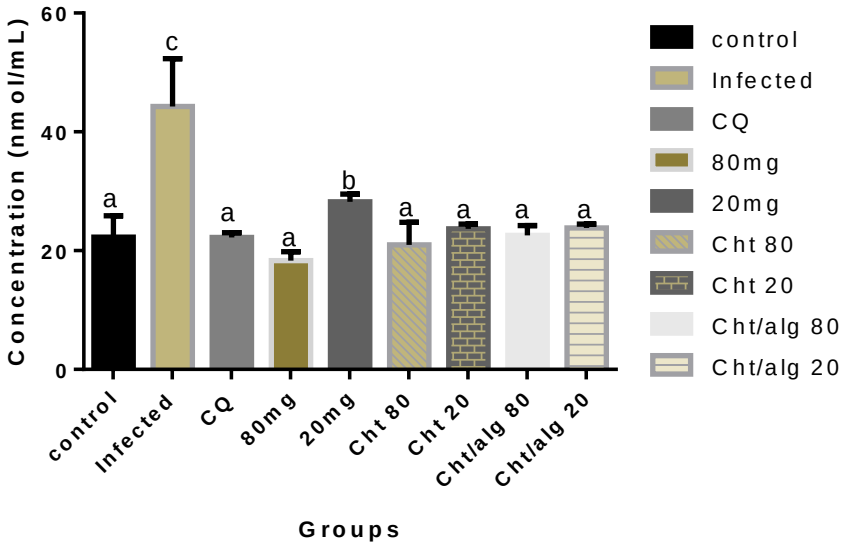

Figure 10: MDA level in liver of mice administered ethylacetate fraction of *Cocos nucifera* bound to chitosan nanoparticles are means± SEM of 5 replicates

Discussion

Several study has reported various part of cocos *nucifera* to have beneficial and medicinal importance, coconut water has been reported to possess cardioprotective (50), renal protective (51), hepatoprotective and antioxidant (31, 52) activities. *cocos. nucifera* endocarp has been demonstrated to possess vaso-relaxant and antihypertensive activities (53). *Cocos. nucifera* husk fibre has been reported to exhibit antimicrobial (18, 22, 54. 55, 56 57, 58), antimalarial (10,11, 16), antitrichomona), and anti-leishmanial (59) activities.

However, this study was aimed at investigating the lipid and antioxidant profile of chitosan bound ethylacetate fraction of *Cocos nucifera* husk fiber in *p. berghei* infected mice. It is known that *plasmodium* parasites are subjected to high levels of oxidative stress during development in host cells, so that their ability to defend themselves against this aggression is critical to their survival (31). The liver is also rich in glycogen, another nutrient for the parasites any perturbation in this power plant will put the parasite in a state of starvation and inhibit formation of merozoites. It had already been shown in 1957 that the liver glycogen of rats infected with *Plasmodium berghei* is depleted (60).

As a result, these parasites have developed several antioxidant defense mechanisms. Additionally, to compensate for the oxidative stress suffered, *plasmodium* reduces its own production of reactive oxygen species and adapts new mechanisms to prevent oxidative damage arising from the host (31, 52).

In the present study, the level of reduced GSH in liver and kidney caused significant increased $p < 0.05$ in non-treated groups compared to the other test groups and controls, the level of GST in liver and kidney caused significant increased $p < 0.05$ in non-treated groups compared the other test groups and controls , the level of MDA in liver and kidney caused significant increased $p < 0.05$ in non-treated groups compared the other test groups and controls, this result agreed with other work that the glutathione cycle is one of the major systems for avoiding the deleterious effects of free radicals.

Total glutathione levels and the overall activity of GST, MDA and GSH were increased due to the infection, which could be due to a detoxification function needed by the increased presence of radical metabolites, such as H_2O_2 and lipid peroxidation products (28, 29, 30), The administered ethylacetate fraction of *cocos nucifera* bound to chitosan microparticles decreased the overall activities of GSH, MDA and GST, and this inhibition could produce a more sensitive environment for parasites to develop oxidative stress linked to the hemoglobin degradation. This could be due to the parasitic clearance leading to decrease in reactive oxygen species generation by the parasite. These biochemical parameters can provide reliable data for the efficacy of drug (31).

This present study, there was a significantly decreased in the level of the non-treated groups as compared to other treated groups in cholesterol, HDL and LDL level while there was significant increase in triacylglyeride level in non – treated groups compared to other test groups and control after the administered ethylacetate fraction of *cocos nucifera* bound to chitosan microparticles In our results are in concordance with reports cited previously with other research work for example more recently in malaria infected children in Cameroon, malaria patients presented significantly lower levels of total cholesterol and HDL cholesterol than control. It is due to the fact that *in vivo* plasmodium continuously diverts cholesterol from hepatocytes and erythrocytes which leads to a lower cholesterol level in malaria patients (61). This study demonstrated that chitosan bound

ethylacetate fraction of *cocos nucifera* husk fiber shows alteration in action of *plasmodium* which can lead to continuously diverts cholesterol from hepatocytes and erythrocytes which leads to a lower cholesterol level in malaria and it lead to production of enzymatic antioxidant defenses to cope with oxygen free radicals and reactive oxygen species

Conclusion

Based on the results obtained in this research, the administration of ethylacetate fraction of *Cocos nucifera husk fibre* bound to chitosan microparticles may boost body's antioxidant systems, which neutralizes the effects of free radicals and was also able to reverse the change in serum lipid profile caused by malaria infection.

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