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Safety assessment and antimalarial potential of chitosan-bound ethylacetate fraction of *Cocos nucifera* husk fiber in *Plasmodium berghei* infected mice

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Abstract

Antimalarial activity of ethylacetate fraction of *Cocos nucifera* bound to a drug delivery carrier- chitosan or chitosan–alginate, was evaluated in this study. The dried pulverized husk fibre was exhaustively extracted with methanol and ethylacetate successively and was bound to chitosan or chitosan–alginate. A 4-day curative antimalarial test was carried out using *Plasmodium berghei* NK65-infected mice to which the ethylacetate extract fraction was administered at doses of 20 mg/kg (Sub-therapeutic dose) and 80 mg/kg body weight (BW) (Therapeutic dose), extract bound to chitosan at the sub-therapeutic dose (STD) and therapeutic dose (TD) as well as extract bound to chitosan–alginate hybrid at STD and TD. Safety assessment of the extract was also evaluated using selected hematological parameters and organ function indices. The extract reduced percentage parasitemia significantly ($p<0.05$), except for the groups treated with the sub-therapeutic dose alone. The ethylacetate fraction bound to chitosan-alginate hybrid showed higher reduction in percentage parasitemia, followed by the rats treated with the chitosan-bound extract at both the STD and TD as compared to other test groups. The results suggest that ethylacetate extract fraction of *Cocos nucifera* husk fibre has partial antimalarial activity at lower doses and can be enhanced by binding it to a suitable drug delivery carrier.

Keyword: *Cocos nucifera*, Drug delivery carriers, Chitosan, Alginate, Sub-therapeutic dose, therapeutic dose

1. Introduction

Malaria is one of the most dreaded human parasitic diseases in the tropics and subtropics, especially in Africa where 81% of cases and 91% of deaths have been estimated to occur,

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with children under five years of age and pregnant women being most severely affected (WHO, 2014). Nigeria accounts for a quarter of all malaria cases in Africa (WHO, 2008), mostly caused by *Plasmodium falciparum* (Adebayo and Krettli, 2011), with an estimated 100 million malaria cases and over 300,000 deaths per year (WHO, 2010).

The continuous spread of *P. falciparum* resistance to antimalarial drugs poses a serious threat to malaria control programs. This, in addition to the high cost of the potent antimalarial drugs, has left the poor masses to be heavily reliant on traditional herbal medicines, which are often affordable and available (Adebayo and Krettli, 2011). 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 (Willcox, 1999). One of such plants is *Cocos nucifera* Linn, commonly referred to as coconut in English. The decoction of the husk fibre of *C. nucifera* is indigenously used for the treatment of malaria, and its efficacy has been scientifically authenticated, with the ethyl acetate extract of the most common variety (West African Tall variety) showing a high degree of antimalarial activity at higher doses which may predispose subjects to hepatotoxicity and nephrotoxicity (Adebayo *et al.*, 2013; Balogun *et al.*, 2014).

The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In most cases (conventional dosage forms), only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biochemical properties. Therefore, developing a drug delivery system that optimizes the pharmaceutical action of a drug while reducing its toxic side effects *in vivo* is a challenging task (Waree, 2003). One approach is the use of colloidal drug carriers that can provide site specific or targeted drug delivery combined with optimal drug release profiles. This study focuses on the delivery of ethylacetate extract fraction of *C. nucifera* with a suitable drug delivery carrier (chitosan) at therapeutic and sub-therapeutic doses, to determine the antimalarial activity of the extract at sub-therapeutic doses, thereby reducing the risk of toxicity from higher doses.

2. Materials and Methods

2.1. Materials

Plant Material

Husk fibres of *C. 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. It was authenticated at the Department of Plant Biology herbarium, where a voucher specimen number was deposited with a voucher number UIH001/508.

Malaria Parasite

Plasmodium berghei (chloroquine sensitive NK 65) were obtained from the Institute for Advanced Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, Nigeria.

Assay Kits and Reagents Used

ALP, AST, ALT, GGT, LDH, Bilirubin, Albumin, Creatinine and Urea kits are products of Randox Laboratories Ltd. Ardmore Diamond Road Crumlin Co Antrium United Kingdom. All other reagents used were of analytical grade and were prepared in glass apparatus using distilled water and suitable solvents.

2.2. Methods

2.2.1 Preparation of Extracts

The extract was prepared as described in a previous study (Adebayo *et al.*, 2013). The dried husk fibers of the *C. nucifera* (West African tall variety) was pulverized to powder. The powder (1000g) was successively extracted with 5L absolute methanol followed by 2.5L ethyl acetate for 72h per solvent. The extracts were filtered using Whatman filter paper No. 1 and then concentrated. The concentrate was exposed to air and residual solvent was allowed to evaporate at room temperature to obtain the dry extract.

2.2.2 Preparation of Chitosan Microparticles

Chitosan microparticles was prepared by the ionic gelation of chitosan solution with anionic Sodium tripolyphosphate (TPP) (Calvo *et al.*, 1997). First, 1g of chitosan was dissolved in 1L 2% (v/v) acetic acid aqueous solution. Then, 2g of TPP was dissolved in 1L distilled water. Subsequently, 10ml of chitosan solution was added drop wisely into 20 ml of TPP solution. Chitosan colloid microparticles was then formed spontaneously under mild agitation at room

temperature on a magnetic stirrer. After 15-20 minutes, the chitosan colloid microparticles was centrifuged at 4000 rpm for 15 min. Then, the supernatant was discarded and the residue was rewashed three times to remove unbound chitosan and re-dispersed in distilled water for further use.

2.2.3 Loading Ethylacetate Fraction of *C. Nucifera* Extract to Chitosan Microparticles

Varied concentration of ethylacetate fraction of *C. nucifera* was dissolved in ethanol. 1ml of the ethylacetate fraction was added to 9ml of 0.1% chitosan. The solution was then added drop wisely into 20ml of TPP and allowed to stir on a magnetic stirrer for 30mins. Chitosan bound ethylacetate colloid microparticles was then formed spontaneously under mild agitation at room temperature on a magnetic stirrer. The colloid formed was centrifuged at 4000rpm for 15mins and the supernatant discarded. The unbound ethylacetate fraction was removed by centrifugation at 4000 rpm for 30 min and the pellet was washed three times with distilled water.

2.2.4 Preparation of Alginate Coated Chitosan Microparticles

Alginate microparticles was 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 4000 rpm for 15mins and the supernatant discarded. The pellet was washed thrice to remove unbound chitosan and alginates.

2.2.5 Antimalarial Studies

A 4-day curative test (Ryley and Peters, 1970) was used with some modifications. Blood was obtained from the tail of a donor mouse (infected with *P. berghei* NK65) with known parasitemia (>15%) into a sample bottle containing 2 mL citrate/glucose solution. The number of infected red blood cells (RBCs) was counted using a hemocytometer. The blood sample was then diluted appropriately with more citrate/glucose solution to obtain an inoculum size of 1×10^5 infected RBCs in 200 µL, which was used to inoculate intraperitoneally each of the outbred Swiss mice on day 0 in each of the phases.

All animals were infected intraperitoneally on Day 0 and left for 72 hours before commencement of treatment. Blood was obtained from the tail of each mouse every other day, starting from day 4 post-inoculation, and was used to prepare blood smears, which were then fixed with methanol, stained with Giemsa, and microscopically examined (Mag. X1000) by counting parasites in 1 000 up to 6 000 erythrocytes. Mortality was monitored daily for 30 d post-inoculation and the mean survival time (MST) for each group was calculated. Percentage parasitemia and percentage reduction in Parasitemia were calculated by following formulae:

$$\% \text{ Parasitemia} = \frac{\text{Total number of infected RBC x 100}}{\text{Total number of RBC}}$$

$$\% \text{ Reduction} = \frac{\text{Initial Parasitemia} - \text{Final Parasitemia}}{\text{Initial Parasitemia}} \times 100$$

Phase 1: Determination of the Most Effective Extract Fraction *of Cocos nucifera.*

Biological Materials

Twenty five (25) male Swiss albino mice weighing between 25g-30g were randomly assigned into five (5) groups, of five (5) mice each. The animals used were obtained from the animal holding unit of University of Ilorin, Ilorin, Kwara State. The animals were housed in standard plastic cages and acclimatized for a period of two weeks. They were maintained under standard conditions and had access to feed and water *ad libitum*.

- **Group A** (Not infected): Administered appropriate volume of distilled water solution.
- **Group B**: Infected with no administration of extract
- **Group C**: Administered 5 mg/Kg body weight of chloroquine.
- **Group D**: Administered 80 mg/Kg body weight of methanolic extract fraction.
- **Group E**: Administered 80 mg/Kg body weight of ethylacetate extract fraction.

Phase 2: Dose Response of the Ethylacetate Extract Fraction of *Cocos nucifera*

- **Animal Grouping**

Twenty (20) albino mice were randomly assigned into four (4) groups, of five (5) mice each. Administration of varying dosage of ethylacetate extract fraction (5, 10, 20 and 40mg/kg) was

done orally for four days post-inoculation (curative test) and 0.2ml of the extract was administered as follows:

- **Group A**: Administered 5 mg/kg body weight of Ethylacetate Extract Fraction
- **Group B**: Administered 10 mg/kg body weight of Ethylacetate Extract Fraction
- **Group C:** Administered 20 mg/kg body weight of Ethylacetate Extract Fraction
- **Group D**: Administered 40 mg/kg body weight of Ethylacetate Extract Fraction

Phase 3: Antimalarial Activity of Sub therapeutic and Therapeutic Dose of Ethylacetate Extract Fraction Coupled with Chitosan Microparticles

- **Animal Grouping**

Forty five (45) Swiss albino 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):** Administered appropriate volume of distilled water solution.
- **Group B (Infected):** Administered appropriate volume of distilled water solution
- **Group C**: Administered 5 mg/Kg body weight of chloroquine (CQ)
- **Group D**: Administered TD of extract fraction (80mg)
- **Group E**: Administered STD of extract fraction (20mg)
- **Group F**: Administered TD of extract fraction coupled with Chitosan (CHT 80)
- **Group G**: Administered STD of extract fraction coupled with Chitosan (CHT 20)
- **Group H**: Administered TD of extract fraction coupled with Chitosan/Alginate (CHT/AlG 80)
- **Group I**: Administered STD of extract fraction coupled with Chitosan/Alginate (CHT/ALG 20)

2.2.6 Preparation of Serum

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. While the blood collected in EDTA bottles were immediately taken for haematological analysis at chemical pathology department of University of Ilorin teaching hospital.

2.2.7 Hematological Analysis

All haematological parameters were estimated using an automated hematological analyzer, SYSMEX-KX21 (SYSMEX Corporation, Japan).

Determination of Concentrations of Selected Serum Biomolecules

Protein concentration was determined by the method of Gornall *et al.,* 1949. Liver function indices such as Serum bilirubin and albumin concentration was determined by the method of Jendrassik and Grof (1938) and Doumas *et al.* (1971) respectively. Kidney function indices such as Serum Creatinine and urea concentrations concentration was determined using the method described by the methods of Bartels and Bohmer (1972), and Veniamin and Vakirtzi (1970) respectively.

2.2.8 Enzyme Assays

The method of Reitman and Frankel (1957) was used to determine Alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST) activity. Alkaline phosphatase activity was determined by the method described by Wright *et al.* (1972). Lactate Dehydrogenase (LDH) and Gamma Glutamyltransferase (GGT) activites was determined by the procedure described by Weisshaar *et al*. (1975) and Szasz (1969) respectively.

2.2.9 Statistical Analysis

Data were expressed as the means ± SEM of 5 determinations. Statistical analysis was performed using One-way Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT). The data were considered statistically significant at $P < 0.05$.

3. Result and Discussion

Phase 1: Determination of the most effective extract fraction of *Cocos nucifera*

Antimalaria Studies

On day 6 and 8 Post innoculation there was a considerable increase in the percentage parasitemia of the untreated group when compared to the groups treated with the reference drug (5mg/kg B.wt CQ) and the test extract dosages (80mg/kg B.wt of the methanolic fraction and 80mg/kg B.wt of the ethylacetate fraction). The reference drug followed by the ethylacetate fraction showed a wide decrease in percentage parasitemia as compared to the mathanolic fraction (Table 1).

Phase 2: Dose response of the Ethylacetate Extract Fraction of *Cocos nucifera*

Antimalaria studies

Percentage parasitemia across all groups decreased progressively with days except for the group treated with 5 mg/kg bw, which showed an increase in percentage parasitemia (Table 2).

Phase 3: Antimalarial Activity of Sub therapeutic and Therapeutic Dose of Ethylacetate Extract Fraction Coupled with Chitosan Microparticles

Antimalaria Studies

The percentage parasitemia decreased across all groups with days except for the untreated group which showed an increase. A high percentage reduction was noticed in the groups treated with the reference drug (CQ), chitosan and the chitosan-alginate hybrid coupled with the therapeutic dose as compared to other test groups.

Haematological Assessment

The untreated group showed a significant decrease ($P<0.05$) in RBC, HCT, MCHC and HGB as compared to other groups. There was no significant difference ($P>0.05$) in WBC, MCH and MCV across all groups.

The antimalarial activity of the methanolic extract and ethylacetate extract of *Cocos nucifera* husk fibre has already been established, but these extracts have only worked at higher doses which predisposes the test animals to hepatotoxicity and nephrotoxicity (Adebayo *et al.*, 2013 and Balogun *et al*., 2014). One way to curb this problem is by reducing the dose administered and delivering it to the target site using natural biodegradable polymers to increase the bioavailability of the drug (Joshi and Patel, 2012).

Earlier reports have indicated the presence of polyphenols in *C. nucifera* husk fibre extract (Mendonça-Filho *et al*., 2004). Polyphenolic contents (such as flavonoids and tannins) of the coconut husk fibre are reported to be responsible for its antimicrobial and antileishmanial activities (Esquenazi *et al*., 2002; Mendonça-Filho *et al*., 2004). Thus, the activities observed in this study may also be due to the presence of such phytochemicals in the extract.

In the first phase of this study the best fraction between the methanolic and the ethylacetate extracts of the *cocos nucifera* husk fiber was determined by a curative antimalarial test. The ethylacetate fraction proved to be more potent by showing a 44% reduction in percentage parasitemia as compared to the methanolic fraction which showed only 36% reduction at a dose concentration of 80mg/kg B.wt. This concentration is said to be the therapeutic dose, because it gave more than 30% reduction in parasitemia, which is in accordance with previous studies (Adebayo *et al.*, 2013; Balogun *et al*., 2014). The ethylacetate fraction was then carried on into the next phase to determine its dose dependent response and a subtherapeutic dose.

The antimalarial study at the second phase demonstrated a dose dependent response in the percentage reduction ($P<0.5$) of parasitemia at different doses of the ethylacetate extract fraction administered (5, 10, 20, and 40mg/kg bw) and the dosage that gave lesser than 30% reduction in percentage parasitemia was considered to be the subtherapeutic dose (Adebayo *et al.*, 2013 and Balogun *et al*., 2014). All doses administered gave lesser than 30% reduction, except for the 40mg/kg bw. Only the 20 mg/kg B.wt was considered in the third phase of the study, for it gave a higher loading efficiency as compared to the 10mg/kg B.wt.

In the third phase, the therapeutic dose and subtherapeutic dose bounded to chitosan and chitosan-alginate microparticles were able to substantially reduce percentage parasitemia, as compared to the unbound ones, although it was still lesser than the chloroquine treated group but it has shown that it is able to increase the bioavailability of the drug at lesser doses and increase the antimalaria activity of the therapeutic dose.

A significant increase ($P<0.05$) in the activities of ALT and AST recorded in the serum of the untreated and the sub-therapeutic dose groups as compared with all the other groups, may be as a result of liver and kidney injury and altered hepatocyte integrity caused by the malaria infection and the consequent release of the enzymes into the blood stream. The increased serum Alkaline Phosphatase (ALP) as recorded in the serum of the untreated and subtherapeutic dose group may be due to: Congestion or obstruction of the biliary tract, which may occur within the liver, the ducts leading from the liver to the gallbladder, or the duct leading from the

gallbladder through the pancreas that empty into the duodenum (small intestine). Any of these organs (liver, gallbladder, pancreas, or duodenum) may be involved (Malbica and Hart, 1971). The recorded increase in serum LDH activity in this study of the untreated group as compared to others can be accounted for by a synergy between the two pathophysiological processes usually associated with acute malaria infections, i.e., the hepatic activity of the invading sporozoites leading to centrilobular liver damage and the destruction of the host red blood cells consequent to erythrocytic merogony (Maegraith, 1981). Being rich sources of LDH, the acute liver injury and red blood cell destruction will be followed by the release of LDH into blood circulation.

In this study, elevated activities of GGT in the serum in correlation with increased ALP activity shows the liver of the animals of the untreated group and the subtherapeutic dose group have been compromised as compared to other groups, which compared favorably with the control group.

Assessment of albumin in the liver could be used to ascertain the secretory and synthetic functions while the level of total bilirubin can be used to monitor the excretory function of the liver (Yakubu *et al.*, 2003). Decrease recorded in the concentration of albumin and a corresponding increase in total of the untreated group as compared to other groups could be as a result of haemolysis of red blood cell.

The increased urea and creatinine concentration in the serum of the untreated and sub-therapeutic dose group may be as a result of the formation Casts loaded with malaria pigment (haemozoin) (Mishra *et al*, 2002). These casts are formed on entry of the malaria parasite into the red blood cell (RBC), which produces changes in the surface of the parasitized cell causing formation of a knob-like process that helps in anchoring the endothelium and adhesion between RBCs. This tight pack of RBCs impedes the microcirculation to various vital organs (Mishra *et al*, 2002). Inability of parasitized RBCs to deform according to the need of microcirculation leads to sluggish blood flow and consequently to renal ischaemia. These casts are also found in the convulated tubules, blocking the clearance of urea and creatinine in the kidney. They are thus being reabsorbed back into the blood and consequently raising blood urea and creatinine levels (Mishra *et al*, 2002). The reduced concentration in other test groups may be as a result of its inhibitory effect on haemozoin formation.

Table 1: Antimalarial Activity of Methanolic and Ethylacetate Extract Fraction of *Cocos nucifera* Husk Fibre

Groups	Percentage parasitemia (%)			Percentage Reduction (%)
	Day 4 PI	Day 6 PI	Day 8 PI	
Untreated	8.05	10.30	15.10	0.00
5mg/kg B.wt CQ	10.60	3.30	2.70	74.0
80mg/kg B.wt Methanolic fraction	8.59	7.50	5.50	36.00
80mg/kg B.wt Ethylacetate fraction	5.10	3.1	2.87	44.00

PI: Post Inoculation Values are percentage means of 5 replicates

Table 2: Dose response of Ethylacetate Extract Fraction of *Cocos nucifera* Husk Fiber

	Percentage parasitemia (%)			
Groups	**Day 4 PI**	**Day 6 PI**	**Day 8 PI**	**Percentage Reduction (%)**
5mg/kg B.wt	10.3	10.9	13.6	0.0
10mg/kg B.wt	10.4	9.4	8.0	23.0
20mg/kg B.wt	8.8	7.3	6.5	26.0
40mg/kg B.wt	10.5	7.2	5.5	35.2

PI: Post inoculation Values are percentage means of 5 replicates

Table 3: Antimalarial activity of Sub therapeutic and therapeutic dose of Ethylacetate Extract Fraction Coupled with Chitosan Microparticles

Percentage parasitemia (%)

Groups	Day 4 PI	Day 6 PI	Day 8 PI	Percentage Reduction (%)
Untreated	5.3	6.4	8.0	0.00
CQ	5.2	2.6	1.5	71.00
80mg	4.7	3.8	2.7	43.00
20mg	5.0	4.1	3.6	28.00
CHT 80mg	5.3	2.8	2.5	53.00
CTH 20mg	4.6	3.9	3.0	37.20
CHT/ALG 80 mg	5.3	2.8	1.7	68.00
CHT/ALT 20mg	4.0	3.6	2.4	40.00

PI: Post Inoculation Values are Parasitemia percentage means of 5 replicates

Table 4: Haematological parameters of *P. berghei* infected mice administered with the Sub-therapeutic and Therapeutic Dose of Ethylacetate Extract Fraction Coupled with Chitosan Microparticles

Groups	WBC ($x10^3/\mu L$)	RBC ($x10^3/\mu L$)	HGB (g/dL)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLT ($x10^3/\mu L$)
Normal	18.1000 ±1.60000^{a}	8.4733 ±0.44122^{b}	12.0667 ±0.95277^{b}	41.7000 ±2.40069^{bc}	49.2333 ±1.04934^{a}	14.2000 ±0.41633^{a}	28.9000 ±0.75056^{bc}	259.6667 ±64.54284^{abc}
Untreated	8.2500 ±0.65000^{a}	3.5400 ±1.67000^{a}	4.4000 ±1.40000^{a}	13.3500 ±1.65000^{a}	55.9000 ±6.70000^{b}	14.8000 ±1.20000^{a}	26.7500 ±01.15000^{a}	527.0000 ±267.0000^{bc}
CQ	2.2000 ±1.88237^{a}	8.4100 ±0.1953^{b}	12.3667 ±0.98376^{b}	41.1000 ±3.66652^{bc}	48.9667 ±0.56075^{a}	14.7667 ±0.28480^{a}	30.1000 ±0.30551^{c}	432.3333 ±129.4737^{abc}
20mg	10.4333 ±3.74448^{a}	9.2733 ±0.44047^{b}	14.5667 ±0.49103^{b}	49.4000 ±1.47309^{c}	53.3667 ±1.31191^{ab}	15.5000 ±0.40415^{a}	29.2667 ±0.17638^{bc}	128.0000 ±38.68247^{a}
80mg	12.9333 ±3.37853^{a}	8.2167 ±0.25719^{b}	11.9333 ±0.60645^{b}	41.0667 ±1.47234^{bc}	50.0333 ±1.82787^{ab}	14.5000 ±0.65574^{a}	29.0333 ±0.43333^{bc}	593.3333 ±224.91505^{c}
CHT 20mg	9.3667 ±0.29627^{a}	8.4900 ±0.44736^{b}	12.0000 ±0.75498^{b}	42.0667 ±2.50954^{bc}	49.5333 ±1.48810^{ab}	14.1333 ±0.28480^{a}	28.5000 ±0.45092^{bc}	163.6667 ±37.10046^{ab}
CHT 80mg	14.3000 ±7.15914^{a}	7.8333 ±0.50446^{b}	11.8333 ±0.72648^{b}	39.6000 ±2.55799^{b}	50.5667 ±1.04775^{ab}	15.1333 ±0.61192^{a}	29.9000 ±0.60277^{bc}	189.6667 ±49.93774^{ab}
CHT/ALG 20mg	11.4000 1.33167^{a}	8.7367 ±0.50824^{b}	12.5000 ±1.23423^{b}	43.7333 ±3.79751^{bc}	49.9000 ±2.00333^{ab}	14.2333 ±0.73106^{a}	28.5000 ±0.37859^{bc}	279.6667 ±83.85371^{abc}
CHT/ALG 80mg	10.1667 ±2.38770^{a}	9.2067 ±0.67844^{b}	13.4333 ±0.91712^{b}	47.5667 ±3.20902^{bc}	51.7667 ±1.14066^{ab}	14.6333 ±0.56075^{a}	28.2667 ±0.44096^{ab}	279.0000 ±32.04684^{abc}

Values are mean of 5 replicates ± SEM, values carrying different superscripts for each parameter are significantly ($P < 0.05$) different.

Enzymes studied

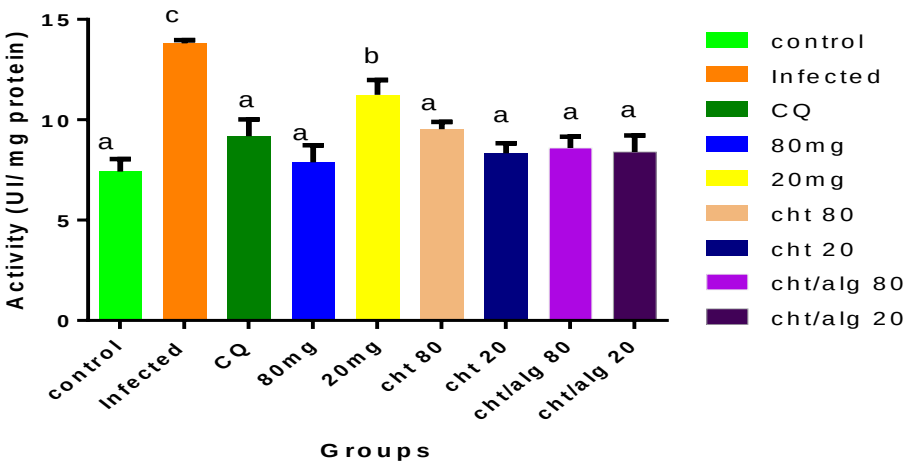

Figure 1: Alanine aminotransferase (ALT) activity in the serum of *P. berghei* Infected Mice Administered with the Sub-therapeutic and Therapeutic Dose of Ethylacetate Extract Fraction Coupled with Chitosan Microparticles.

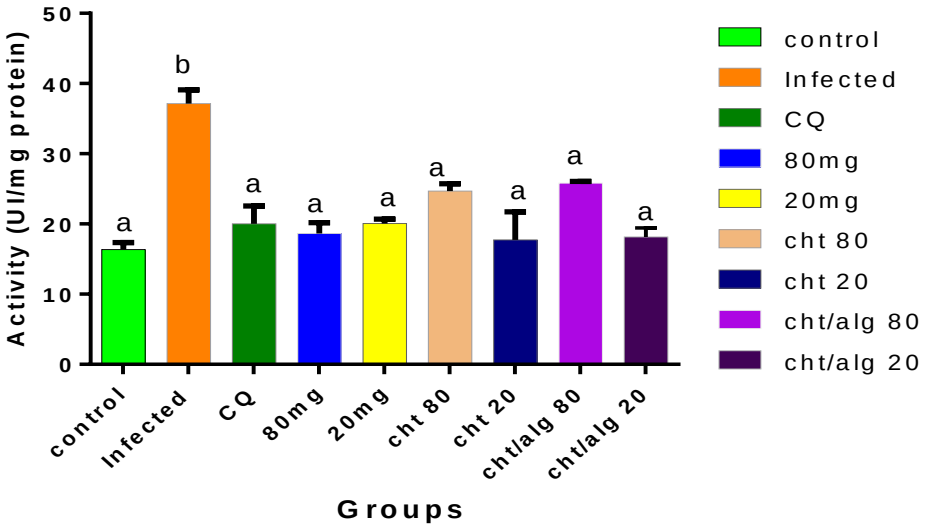

Figure 2: and Aspartate aminotransferase in the serum of *P. berghei* Infected Mice Administered with the Sub-therapeutic and Therapeutic Dose of Ethylacetate Extract Fraction Coupled with Chitosan Microparticles.

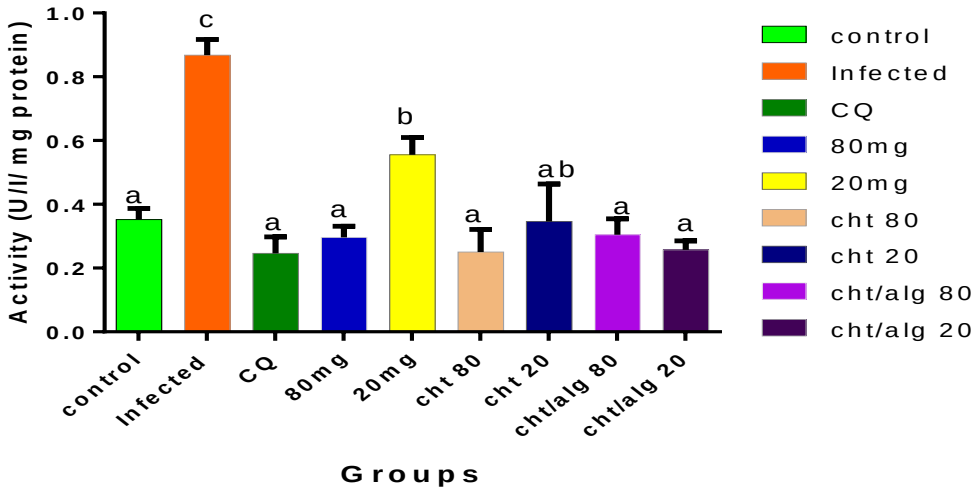

Figure 3: Alkaline phosphatase (ALP) activity in the serum of *P. berghei* Infected Mice Administered with the Sub-therapeutic and Therapeutic Dose of Ethylacetate Extract Fraction Coupled with Chitosan Microparticles.

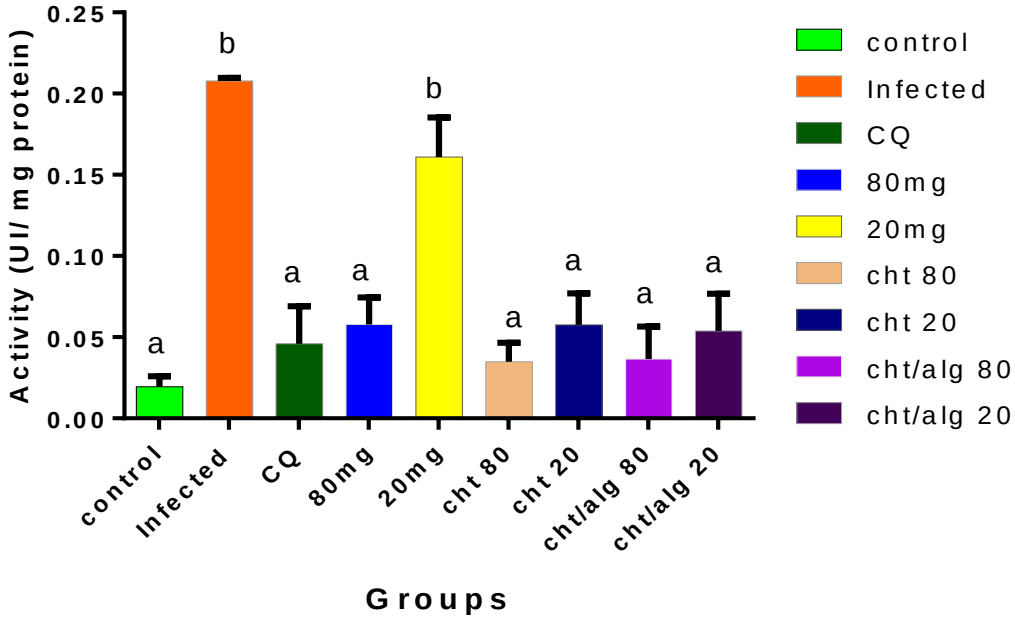

Figure 4: Lactate Dehydrogenase (LDH) activity in the serum of *P. berghei* Infected Mice Administered with the Sub-therapeutic and Therapeutic Dose of Ethylacetate Extract Fraction Coupled with Chitosan Microparticles.

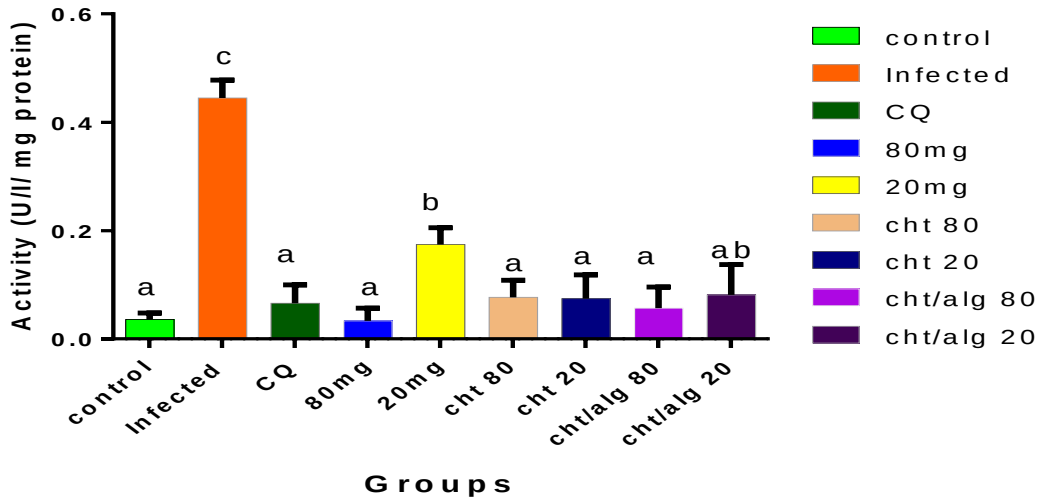

Figure 5: Gamma glutamyltranferase (GGT) activity in the serum of *P. berghei* Infected Mice Administered with the Sub-therapeutic and Therapeutic Dose of Ethylacetate Extract Fraction Coupled with Chitosan Microparticles.

Liver Function Indices

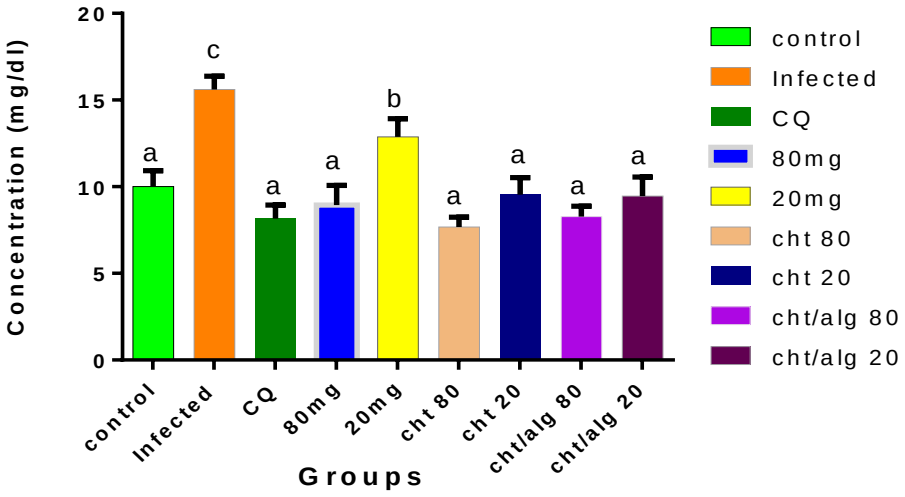

Figure 6: Total bilirubin concentration in the serum of *P. berghei* Infected Mice Administered with the Sub-therapeutic and Therapeutic Dose of Ethylacetate Extract Fraction Coupled with Chitosan Microparticles.

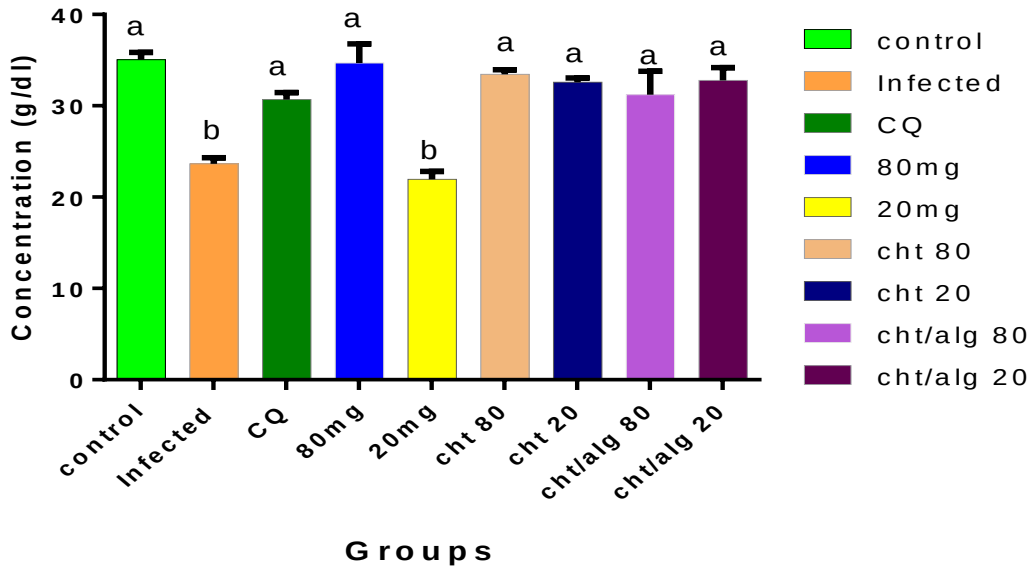

Figure 7: Albumin concentration in the serum of *P. berghei* Infected Mice Administered with the Sub-therapeutic and Therapeutic Dose of Ethylacetate Extract Fraction Coupled with Chitosan Microparticles.

Kidney Function Indices

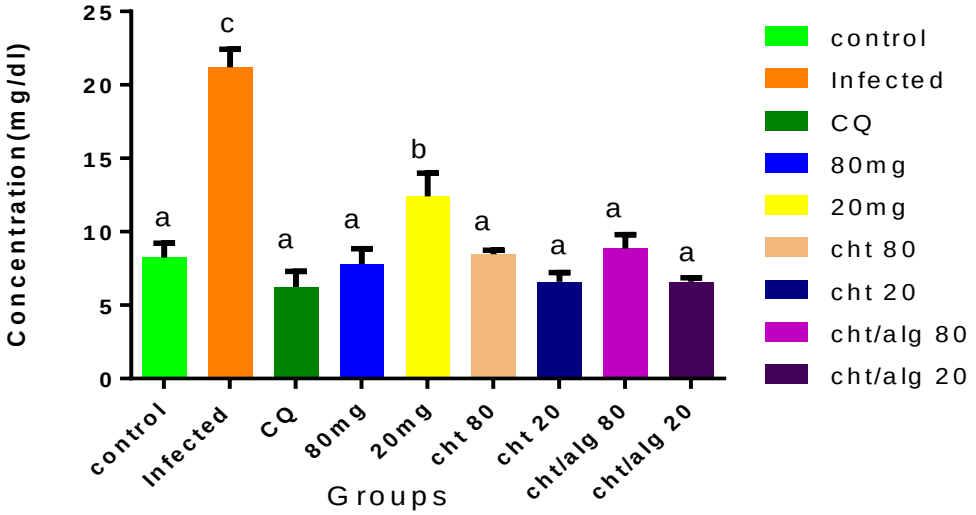

Figure 8: Creatinine concentration in the serum of *P. berghei* Infected Mice Administered with the Sub-therapeutic and Therapeutic Dose of Ethylacetate Extract Fraction Coupled with Chitosan Microparticles.

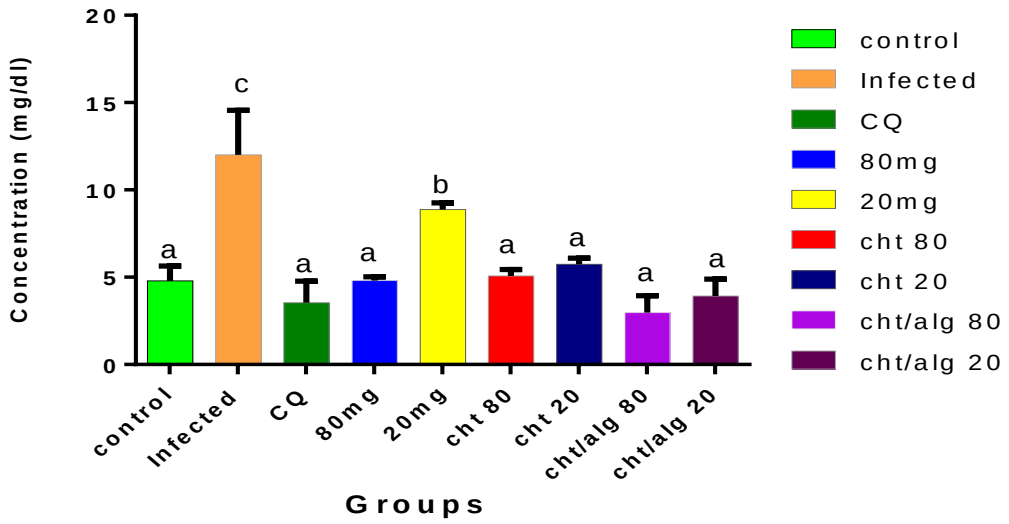

Figure 9: Urea concentration in the serum of *P. berghei* Infected Mice Administered with the Sub-therapeutic and Therapeutic Dose of Ethylacetate Extract Fraction Coupled with Chitosan Microparticles.

4. Conclusion

The result of this study shows that the enhancement of antimalaria activity of ethylacetate fraction of *Cocos nucifera* husk fiber at lower doses when bound to a drug delivery carrier. There was enhancement of antimalaria activity and reduced toxicity at a higher dose. There was reversal of malaria mediated alterations in the liver and kidney function indices (ALP, AST, ALT, GGT, LDH, Albumin, Bilirubin, Urea and Creatinine). Thus, from the findings of this study, it is evident that chitosan bound to the ethylacetate fraction of *C. nucifera* husk fiber is generally safe for administration and can be adopted for other studies as means of delivering drugs at low doses.

Recommendation

This study thus supports the recommendation of using chitosan as a means of delivering drugs at low doses as this could help to reduce toxicity and repeated administration of drugs over prolonged periods.

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