Ameliorative effects of some phytochemicals extracted from Cola acuminate leaf on altered liver function indices of mice infected with Plasmodium berghei (NK-65)

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Abstract- Alterations in liver function indices are among classic features of malaria complications resulting from activities of the parasite in the liver. *Cola acuminata* has been reported to contain phytochemicals with antimalarial activity. This study assessed the effects of some of these phytochemicals on liver function indices of *Plasmodium berghei* infected mice. For each phytochemical, 7 groups (A-G) of five mice each were used. Group A served as normal control, B-F were inoculated with *Plasmodium berghei*; group B served as untreated control while groups C-F were treated with 20mg/Kg body weight chloroquine, 12.5, 25 and 50 mg/kg body weight of the different phytochemicals respectively. Group F was treated with 50mg/Kg body weight of phytochemicals only without parasite inoculation thus serving as extract control. Treatment commenced 72hrs after inoculation; once orally for four consecutive days. A week after treatment, the mice were sacrificed under diethyl anesthesia and blood was collected for evaluation of liver function indices. Liver function indices including total bilirubin, albumin, total protein, the transaminases and alkaline phosphatase were evaluated. Infection with *Plasmodium berghei* caused a progressive increase in total bilirubin concentration as well as the activities of AST, ALT, and ALP and decrease in total protein and albumin concentration. Treatment with all phytochemical extracts improved liver function indices in a dose-dependent manner comparable to the normal group at the highest dose. Thus, they can be a promising source of effective antimalarial therapy individually or in combined therapy.

Index Terms- Cola nitida, phytochemicals, liver function indices, Plasmodium berghei

I. Introduction

Hepatic involvement in malaria is a common presentation, and presence of jaundice is one of the indicators of severe malaria as defined by World Health Organization in 2008. Malaria infection results in systemic manifestation with effects on vital organs like kidney and liver (Adelakun *et al.,* 2015). The effects of this infection on the liver are attributed to the hepatic stage of the life cycle of the parasite in the host which takes place in the liver (Zailani *et al.,* 2020). The sporozoite invasion of hepatocytes during the exo-erythrocytic stage of malaria parasite life cycle contributes immensely to liver dysfunction in malaria. The presence of sporozoites in the liver during exo-erythrocytic as well as accumulation of haemozoin in the liver during erythrocytic stages of malaria parasite life cycle induces immune-mediated damages to the hepatocytes. This causes leakage of parenchymal and membranous enzymes of the liver into the general circulation which might be responsible for the increase in serum activities of these liver marker enzymes. The alteration in the liver function markers have been found to correlate with parasite density as well as the severity of the infection (Enechi *et al.,* 2019). As reported in previous studies, the liver damage manifests as a sudden increase in serum activities of liver marker enzymes in malaria infected individuals (Jarikre *et al., 2001*; Onyesom and Onyemakonor, 2011). On the other hand, clinical trials of novel antimalarial drugs have reported abnormalities in liver enzymes, and/or total bilirubin (TB) (Cheaveau *et al*., 2019). Plant materials used in sub-saharan Africa (including *Cola acuminata* leaf) for the treatment of malaria have been found to contain active substances that exhibit antimalarial activity either individually or in combination (Zailani *et al*., 2016; Eromosole and Kehinde, 2018; Zailani *et al*., 2020) . This work was aimed at evaluating the effects of some phytochemicals extracted from *Cola acuminata* leaf on liver function parameters of *Plasmodium berghei* (NK-45) infected mice.

II. Materials and Methods

2.1 Collection and identification of plant material

Fresh leaf samples of *Cola acuminata* was collected from Gembu, Sardauna Local Government of Taraba State, Nigeria. The leaves were identified and authenticated at the Department of Plant Sciences, Modibbo Adama University Technology Yola, Adamawa State.

2.2 Experimental Animals

Seventy (70) Swiss albino mice (about 6 to 8 weeks old) were obtained from the animal breeding unit of the University of Jos, Plateau State. The average body weight of mice (22.02±1.37g) was measured using a Shimadzu (UX4200H) top pan animal balance to the nearest 0.1g. The mice were housed in

plastic cages and maintained under standard laboratory conditions with free access to rat pellets and tap water *ad libitum*.

2.3 Parasite Strain

Chloroquine sensitive strain of *Plasmodium berghei* (NK-65) was obtained from Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Science, Ahmadu Bello University Zaria, Kaduna State Nigeria.The parasites were maintained by weekly serial passage of blood from the donor-infected mice to healthy uninfected mice via intraperitoneal (IP) injection (Ounjaijean, *et al*. 2019)

2.4 Chemicals and reagents

Chloroquine diphosphate salt, immersion oil, and Giemsa strain were obtained from Sigma Chemical Company St. Louis, Mo, USA. Assay kits for enzymes and liver function indices were obtained from Randox Laboratory Ltd, UK.

2.5 Preparation of plant sample

Whole fresh leaves of *Cola acuminata* were washed with water and dried in the shade at room temperature. The dried leaf sample was ground to powder using an electric blender (Mazeda Mill, MT 4100, Japan). It was then packed in a sealed plastic bottle until extraction.

2.6 Extraction of flavonoids

Flavonoids were extracted according to the method described by Yahaya, (2015).

2.7 Extraction of phenolics

Phenolics were extracted according to the method described by Velioglu *et al.* (1998)

2.8 Parasite inoculation

The mice were inoculated from the same donor mouse. Each mouse was inoculated intraperitoneally on day 0 with 0.2 ml of infected blood containing about 1×10^7 *Plasmodium berghei* parasitized red blood cells. They were then monitored for 72 hours after which infection was confirmed by observing tail blood microscopically before treatment was started.

2.9 Extract and chloroquine administration

Experimental groups to receive extract or the standard drug (chloroquine) started receiving treatment 72 hours after infection. The administration of the extract as well as the standard drug was carried out orally using an intra-gastric tube and treatment was maintained daily for 4 days.

2.10 Experimental design

For each phytochemical, the mice were divided into seven groups (A-G) of five mice per group.

Group A was not inoculated and not treated and served as normal; group B was inoculated but untreated and served as infected control. Group C was treated with 20mg/Kg body weight of chloroquine (treated control). Groups D, E and F were inoculated with *Plasmodium berghei* (pb) and administered 12.5, 25 and 50 mg/kg body weight of the different phytochemicals respectively while group F was treated with 50mg/Kg body weight of the phytochemicals only without parasite inoculation thus

serving as extract control. A week after treatment, the mice were sacrificed and blood collected to evaluate the effect of treatment on liver function indices.

III.   DETERMINATION OF LIVER FUNCTION INDICES AND ASSAY OF SOME ENZYMES

A week after treatment, the mice were sacrificed after diethyl ether anesthesia and blood was collected in plain sterile containers and centrifuged for 5 minutes at 2500rpm using Wisperfuge centrifuge (model 1384, Samson, Holland) to obtain serum. The serum was then collected for the assay of liver function markers as indicated below:

3.1 Determination of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP) activities.

These were done as described by Reitman and Frankel, (1957).

3.2 Determination of Bilirubin concentration

This was determined using the calorimetric method of White *et al*. (1958).

3.3 Determination of Total Protein concentration

The method of Tietz, (1976) was adopted in the determination of serum total protein concentration.

3.4 Determination of Albumin concentration

The method described by Doumas *et al*.(1971) was adopted to determine serum albumin concentration.

IV.   STATISTICAL ANALYSIS

The grouped data was expressed as mean ± standard error of mean (SEM) and the significant differences were determined using Statistical Package for Social Sciences (SPSS V. 25).

V.   RESULTS

Table 1 shows the results of the effects of some phytochemicals extracted from *Cola acuminata* leaf on the activities of some liver enzymes in mice infected with *Plasmodium berghei* (NK-65). Infection of the mice with *Pasmodium berghei* significantly ($P<0.05$) increased the activity of the enzymes studied. Treatment of the infected mice with chloroquine and the extracts resulted in significant ($P<0.05$) improvement of the distorted indices towards normal in a dose dependent manner. Results obtained for the extract control group showed that the phytochemicals did not cause any significant changes in the activities of these enzymes compared to normal.

Table 2 shows the effects of some phytochemicals extracted from *Cola acuminata* leaf on some liver function indices of *Plasmodium berghei* (NK-65) infected mice. Infection of the mice with the parasite significantly ($P<0.05$) decreased total protein and albumin concentrations while significantly ($P<0.05$) increasing total bilurubin concentration when compared with the normal control group. Treatment of the infected mice with

chloroquine and all the phytochemical extracts significantly improved (P<0.05) all the function indices towards normal in a dose dependent manner. Similarly, the extract control group did not elicit any significant changes in the function indices at the highest dose compared to normal control.

RESULTS

Table 1: Effects of flavonoids and phenolics extracted from *Cola acuminata* leaf on some liver enzymes in mice infected with *Plasmodium berghei* (NK-65).

PHYTOCHEMICALS	TREATMENT	AST(IU/L)	ALT(IU/L)	ALP(IU/L)
FLAVONOIDS	Normal control	12.87±1.70[a]	50.75±3.59[a]	63.75±4.00[a]
	untreated control	41.90±1.04[b]	83.25±6.36[b]	82.60±1.22[b]
	treated control 20mg/kg b.w.t of chloroquine	11.78±0.65[a]	52.22±2.06[a]	65.32±3.48[a]
	12.5mg/kg b.w.t of extract + pb	35.05±0.73[c]	75.00±8.23[c]	76.75±5.69[c]
	25mg/kg b.w.t of extract + pb	14.98±0.67[a]	54.10±3.00[a]	70.02±8.22[a]
	50mg/kg b.w.t of extract + pb	12.77±1.98[a]	52.75±2.43[a]	68.72±7.41[a]
	50mg/kg b.w.t of extract without pb	12.50±0.99[a]	51.90±2.05[a]	64.27±3.03[a]
PHENOLICS	Normal control	9.50±0.99[a]	50.75±3.59[a]	68.72±7.41[a]
	untreated control	51.90±1.04[d]	103.25±6.36[d]	162.60±1.22[d]
	treated control 20mg/kg b.w.t of chloroquine	11.78±0.65[a]	54.75±2.42[a]	83.75±4.17[a]
	12.5mg/kg b.w.t of extract + pb	33.15±1.35[c]	73.25±3.96[c]	115.75±9.30[c]
	25mg/kg b.w.t of extract + pb	26.20±2.31[b]	64.75±4.13[b]	95.25±4.83[b]
	50mg/kg b.w.t of extract + pb	14.65±0.73[a]	53.25±2.53[a]	84.60±4.12
	50mg/kg b.w.t of extract without pb	10.40±0.93[a]	51.50±2.25[a]	69.99±8.27[a]

Values are means of 5 replicates± SEM. Means in the same column with different superscripts are significantly different (P<0.05).   pb = *Plasmodium berghei*

Key: AST= aspartate transaminase, ALT= alanine transaminase, ALP=alkaline phosphatase.

Table 2: Effects of flavonoids and phenolics extracted from *Cola acuminata* leaf on some liver function indices of *Plasmodium berghei* (NK-65) infected Mice.

PHYTOCHEMICALS	TREATMENT	TP(mg/mL)	ALB(mg/mL)	TB(mg/mL)
FLAVONOIDS	Normal control	89.33±1.12[a]	37.65±1.74[a]	2.77±0.20[a]
	untreated control	48.85±2.93[b]	27.97±1.41[b]	9.73±0.84[b]
	treated control 20mg/kg b.w.t of chloroquine	86.05±6.61[a]	35.60±1.32[a]	2.83±0.55[a]
	12.5mg/kg b.w.t of extract + pb	52.33±1.36[c]	28.92±1.69[b]	6.37±1.02[c]
	25mg/kg b.w.t of extract + pb	82.65±4.20[a]	30.10±2.00[a]	3.47±0.95[a]
	50mg/kg b.w.t of extract + pb	85.10±5.41[a]	33.32±2.38[a]	2.90±0.38[a]
	50mg/kg b.w.t of extract without pb	84.20±6.22[a]	35.32±2.35[a]	2.81±0.54[a]
PHENOLICS	Normal control	89.33±1.12[a]	37.65±1.75[a]	2.77±0.20[a]

	untreated control	48.85±2.93[d]	27.00±0.71[d]	9.73±0.84[d]
	treated control 20mg/kg b.w.t of chloroquine	85.58±1.63[a]	34.50±1.75[a]	4.73±0.55[a]
	12.5mg/kg b.w.t of extract + pb	51.75±1.25[c]	30.55±1.13[c]	8.93±0.78[c]
	25mg/kg b.w.t of extract + pb	60.58±0.74[b]	31.45±1.25[b]	6.33±0.56[b]
	50mg/kg b.w.t of extract + pb	83.46±1.32[a]	33.66±1.26[a]	4.88±0.71[a]
	50mg/kg b.w.t of extract without pb	91.05±2.93[a]	39.65±1.75[a]	3.01±0.86[a]

Values are means of 5 replicates± SEM. Means in the same column with different superscripts are significantly different (P<0.05).
Key: TP=total protein, ALB= albumin, Pb = *Plasmodium berghei*

VI. DISCUSSION

Malaria infection has been reported to involve liver damage (WHO, 2008; Adelakun *et al.,* 2015; Zailani *et al.,* 2020) which manifests in the activities of liver function enzymes (alanine transaminase ALT, aspartate transaminase AST as well as alkaline phosphate ALP) and haemolysis which leads to alterations in the normal bilurubin concentration (Adelakun *et al.,* 2015; Zailani *et al.,* 2020). These reports are supported by findings in this study (Tables 1 and 2). These alterations, usually associated with the sporozoite invasion of hepatocytes during the exo-erythrocytic stage of malaria parasite life cycle contribute immensely to liver dysfunction in malaria (Onyesom, 2012). The presence of sporozoites in the liver during the exo-erythrocytic stage as well as accumulation of haemozoin in the liver during erythrocytic stages of malaria parasite life cycle induces immune-mediated damage to the hepatocytes which causes leakage of parenchymal and membranous enzymes of the liver into general circulation, which might be responsible for the increase in serum activities of these liver marker enzymes as observed (Burtis and Ashwood, 2001). Increased haemolysis on the other hand was reported to result in the elevation of bilirubin concentration (Adelakun, *et al.*, 2015). Previous findings reported by Zailani *et al*. (2016) and Zailani *et al*. (2020) asserted that some phytochemicals of *Cola species* exihibit antimalarial activities.

Results in Table 1 shows that the treatment groups significantly (P<0.05) improved the altered enzyme activities towards normal in a dose dependent manner. These findings can be attributed to the antimalarial properties of the phytochemicals previously reported by Zailani *et al*. (2016), membrane stabilization and maintenance of hepatocyte integrity potentials (Oyedapo and Famurewa 1995), by preventing the leakage of liver enzymes (AST, ALT and ALP) into the circulatory system or antioxidant activity of these phytochemicals which reduces hepatic oxidative stress (Momoh and Longe, 2014; Zailani *et al.,* 2016). All these suggest that these phytochemicals possess hepatocurative and ameliorative activities on hepatic dysfunction induced by malaria infection.

Malaria parasites require various host factors for their development and survival. These factors include purines as they are not able to synthesize them *de novo,* iron and amino acids from the host haemoglobin and extracellular milieu as haemoglobin does not contain isoleucine and is low in several amino acids, such as methionine (Tougan, *et al.,* 2020). This is a possible reason for the significant (P<0.05) reduction in the plasma concentrations of albumin and total protein in the untreated control group (Table 2). Significant improvement in the deranged indices in all the treatment groups of the phytochemicals in a dose dependent manner suggests that the extracts were able to improve liver function during malaria infection possibly due to parasite clearance (Chierrito *et al.,* 2014; Zailani *et al.,* 2016). Understanding the mechanisms by which these phytochemicals act facilitates the development of novel antimalarial drugs and also helps in understanding the mechanism of parasite resistance. Some of these phytochemicals have been reported to selectively clear these parasites both *in vivo* and *in vitro* (Tajuddeen and Van Heerden, 2019). Parasite clearance by these phytochemicals may be due to one or more mechanisms employed by these phytochemicals. Terpenoids for example act by inhibiting the growth phase of the *plasmodium* parasite from ring form to tropozoites and inhibited nutrient intake by the parasites by inhibiting the permeation pathway. Flavonoids on the other hand work by inhibiting the influx of L-glutamine and myoinosol into the infected red blood cell. Anthraquinone could kill the parasites through various mechanisms, resulting from aldehyde at C-2 (Umar, *et al.*, 2017). Some phenolics act through detoxification of haem by inhibiting β-haematin formation. Alkaloids act on protein metabolism I the parasite by inhibiting chaperon and ATPase as well as cytoplasmic lysl-tRNA synthatase functions (Tajuddeen and Van Heerden, 2019).

VII. CONCLUSION

All the phytochemicals in this study improved the distorted liver function indices as well as plasma protein concentrations induced by *Plasmodium berghei* infection. This can be attributed to the parasite clearance and antioxidant activities of this extracts as reported in previous literature. Therefore, these phytochemicals can be promising sources or a component of antimalarial agent either individually or in combined therapy.

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