



ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
www.plantsjournal.com
JMPS 2020; 8(2): 140-143
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Received: 21-01-2020
Accepted: 22-02-2020

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Cola nitida leaf phytochemicals improve liver function indices of mice infected with *Plasmodium berghei* (NK-65)

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Abstract

Derangement in liver function indices are among the classic clinical features of malaria infection and are responsible for significant morbidity and mortality. *Colanitida* leaf has been reported to possess antimalarial activity and also contain pharmacologically active phytochemicals including alkaloids, flavonoids, tannins and phenolics. This study evaluated the effects of these phytochemicals on liver function indices of *Plasmodium berghei* (NK-65) infected mice. For each phytochemical, 7 groups (A-G) of five mice each were used. Group A served as normal, groups B-F were inoculated with *Plasmodium berghei*; group B served as untreated control while groups C-F were treated with 20mg/Kg body weight of chloroquine and 12.5, 25 and 50 mg/kg body weight of the different phytochemicals respectively. Group G was treated with 50mg/Kg body weight of the phytochemicals only without parasite inoculation thus serving as extract control. Treatment commenced 72hrs after inoculation and was done once orally for four consecutive days. A week after treatment, 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 level as well as the activities of AST, ALT, and ALP and a marked decrease in total protein and albumin. Treatment with all the phytochemical extracts improved liver function indices in a dose-dependent manner comparable to the normal group. These phytochemicals were all able to restore the *Plasmodium berghei* induced changes in liver function indices probably through parasite clearance and/or antioxidant activities. Thus, they can be a promising source of effective antimalarial drugs individually or in combined therapy.

Keywords: *Cola nitida*, liver function indices, *Plasmodium berghei*

1. Introduction

Perturbations in liver function indices have been reported to be one of the clinical features of malaria infection responsible for significant morbidity and mortality [1]. The pathogenesis of these liver indices though multifactorial, has been attributed to the extra-erythrocytic lifecycle of the malaria parasite that takes place in the liver, cytoadherence of parasitized erythrocytes and products of red blood cell degradation such as free radicals which are harmful to the liver [2, 3, 1, 4]. Reports of malaria-associated liver injury in literature include biochemical assessment of liver function tests (LFTs), which include bilirubin levels and the activity of transaminases (alanine transaminase (ALT) and aspartate transaminase (AST) activities) [4] and albumin concentration [1]. Variation in the liver function indices can be attributed to the individual variation in inflammatory response and the efficiency of heme breakdown. Malarial hepatopathy has been reported in 2.6–45% of all malaria cases and up to 87.5% of cases presenting with clinical jaundice [5] which has been described as a bilirubin level above 2.5 times upper limit of normal (ULN) with associated transaminase elevation above 3 times the ULN (where ALT is considered the more liver-specific enzyme) [6, 7]. On the other hand, clinical trials of novel antimalarial drugs have reported abnormalities in liver enzymes, and/or total bilirubin (TB) [8]. Plants found in sub-saharan Africa including *Cola nitida* have been reported to be used in orthodox medicine for the treatment and management of infections including malaria [9, 10] and have been also found to possess relatively low toxicity [11]. The present work aimed at evaluating the effects of some phytochemicals extracted from *Cola nitida* leaf on liver function indices in *Plasmodium berghei* infected mice.

2. Materials and Methods

2.1.1 Collection and identification of plant material

Fresh leaf samples of *Cola nitida* was collected from Mubi, Mubi Local Government of Adamawa state, Nigeria.

The leaves were identified and authenticated at the Department of Plant Sciences, Modibbo Adama University Technology Yola, Adamawa State.

2.1.2 Experimental Animals

One hundred and forty (140) 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 ± 1.37 g) 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*.

2.1.3 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 [12].

2.1.4 Chemicals and reagents

Chloroquine diphosphate salt, immersion oil, and Giemsa stain 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.

2.2 method

2.2.1 Preparation of plant sample

Whole fresh leaves of *Cola nitida* 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.

2.2.2 Extraction of alkaloids

Alkaloids were extracted gravimetrically according to the method described by [13].

2.2.3 Extraction of flavonoids

Flavonoids were extracted according to the method described by [14].

2.2.4 Extraction of phenolics

Phenolics were extracted according to the method described by [15].

2.2.5 Extraction of Tannins

Extraction of tannins was done according to the method of [16].

2.2.6 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.

2.2.7 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.

2.2.8 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* and administered 12.5, 25 and 50 mg/kg body weight of the different phytochemicals respectively while group G 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.

2.3 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 a plain sterile container 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:

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

These were done as described by [17].

2.3.2 Determination of Bilirubin concentration

Calorimetric method was used based on the method of [18].

2.3.3 Determination of Total Protein concentration

Method of [19] was adopted in the determination of total protein concentration.

2.3.4 Determination of Albumin concentration

The method described by [20] was adopted to determine albumin concentration.

2.4 Statistical Analysis

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

3. Results

The effects of some phytochemicals extracted from *Cola nitida* leaf on some liver enzymes in mice infected with *Plasmodium berghei* (NK-65) is presented in Table 1. Infection of the mice with the parasite was found to significantly ($P < 0.05$) increase the activities of all the enzymes in the untreated control compared with normal control. Treatment of the infected mice with chloroquine and all the phytochemical extracts significantly ($P < 0.05$) improved the activities of these enzymes towards normal. For the extracts, the improvement was dose dependent. The results also showed that administration of the different extracts at the highest dose to normal animals did not significantly ($P < 0.05$) alter the indices compared to the normal control group.

The effects of the phytochemicals extracted from *Cola nitida* leaf on some liver function indices of *Plasmodium berghei*

(NK-65) infected mice is presented in Table 2. Infection with parasite significantly ($P<0.05$) decreased total protein and albumin concentrations while significantly ($P<0.05$) increasing total bilirubin 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.

4. Results

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

Phytochemicals	Treatment	AST(IU/L)	ALT(IU/L)	ALP(IU/L)
	Normal control	15.01±2.84 ^b	59.97±0.84 ^a	77.94±1.38 ^a
Negative control	64.08±0.30 ^f	84.42±1.11 ^d	91.93±0.95 ^d	
Standard control 20mg/kg b.w.t of extract	11.72±0.23 ^a	59.85±0.36 ^a	80.64±0.26 ^{ab}	
Alkaloids	12.5mg/kg b.w.t of extract + MP	61.28±0.55 ^c	82.27±0.52 ^d	86.02±0.56 ^c
	25mg/kg b.w.t of extract + MP	38.45±0.85 ^d	77.38±0.85 ^c	81.62±1.06 ^b
	50mg/kg b.w.t of extract + MP	23.62±0.69 ^c	63.03±0.88 ^b	77.64±1.27 ^a
	50mg/kg b.w.t of extract without MP	15.57±0.56 ^b	60.56±0.88 ^a	79.12±0.58 ^{ab}
Flavonoids	12.5mg/kg b.w.t of extract + MP	59.26±0.44 ^d	81.51±0.35 ^d	88.11±0.54 ^d
	25mg/kg b.w.t of extract + MP	30.53±0.59 ^c	75.03±0.70 ^c	84.42±0.40 ^c
	50mg/kg b.w.t of extract + MP	17.31±0.35 ^b	62.46±0.68 ^b	78.88±0.5 ^{ab}
	50mg/kg b.w.t of extract without MP	14.95±1.26 ^a	63.15±0.34 ^b	78.25±0.34 ^a
Phenolics	12.5mg/kg b.w.t of extract + MP	60.10±0.09 ^e	81.35±0.28 ^d	87.35±0.55 ^d
	25mg/kg b.w.t of extract + MP	30.92±0.61 ^d	75.90±0.54 ^c	61.04±0.46 ^a
	50mg/kg b.w.t of extract + MP	17.91±0.27 ^c	62.52±0.77 ^b	75.90±0.79 ^b
	50mg/kg b.w.t of extract without MP	15.60±0.93 ^b	60.32±1.30 ^{ab}	77.80±1.24 ^b
Tannins	12.5mg/kg b.w.t of extract + MP	56.3320±1.5 ^d	80.7800±0.7 ^d	88.6820±0.51 ^d
	25mg/kg b.w.t of extract + MP	33.0040±0.8 ^c	73.3260±1.55 ^c	84.9140±0.51 ^c
	50mg/kg b.w.t of extract + MP	24.0240±0.6 ^b	61.7720±0.3 ^b	80.1600±0.58 ^{ab}
	50mg/kg b.w.t of extract without MP	22.1260±2.2 ^b	58.5360±0.97 ^a	78.7220±0.98 ^{ab}

Values are means of 5 replicates± SEM. Means in the same column with different superscripts are significantly different ($P<0.05$).

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

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

Phytochemicals	Treatment	TP(mg/mL)	ALB(mg/mL)	TB(mg/mL)
	Normal control	80.30±0.46 ^e	38.52±0.20 ^f	2.36±0.16 ^a
Negative control	35.21±1.78 ^a	25.31±0.36 ^a	7.82±0.42 ^d	
Standard control 20mg/kg b.w.t of extract	76.68±0.49 ^d	36.50±0.39 ^d	3.03±0.11 ^a	
Alkaloids	12.5mg/kg b.w.t of extract + MP	46.90±0.49 ^b	28.23±0.49 ^b	4.56±0.22 ^d
	25mg/kg b.w.t of extract + MP	61.23±0.71 ^c	32.16±0.45 ^c	3.07±0.26 ^c
	50mg/kg b.w.t of extract + MP	71.02±1.09 ^d	37.04±0.56 ^d	2.25±0.13 ^a
	50mg/kg b.w.t of extract without MP	79.16±0.54 ^{ef}	38.58±0.33 ^e	2.14±0.29 ^a
Flavonoids	12.5mg/kg b.w.t of extract + MP	45.15±0.25 ^b	27.65±0.39 ^b	4.63±0.24 ^c
	25mg/kg b.w.t of extract + MP	57.26±2.19 ^c	31.57±0.73 ^c	3.99±0.26 ^b
	50mg/kg b.w.t of extract + MP	74.71±0.95 ^d	35.94±0.37 ^d	2.88±0.13 ^a
	50mg/kg b.w.t of extract without MP	77.68±0.68 ^d	37.75±0.18 ^{ef}	2.30±0.18 ^a
Phenolics	12.5mg/kg b.w.t of extract + MP	49.41±0.43 ^b	28.68±0.28 ^b	4.22±0.27 ^c
	25mg/kg b.w.t of extract + MP	61.04±0.46 ^c	32.40±0.39 ^c	3.12±0.92 ^b
	50mg/kg b.w.t of extract + MP	76.39±0.70 ^d	35.17±0.39 ^d	2.36±0.13 ^a
	50mg/kg b.w.t of extract without MP	65.26±2.77 ^{de}	37.32±0.72 ^{ef}	2.34±0.17 ^a
Tannins	12.5mg/kg b.w.t of extract + MP	43.56±0.75 ^b	27.54 ± 0.27 ^b	4.51 ± 0.16 ^d
	25mg/kg b.w.t of extract + MP	57.96±0.47 ^c	30.30 ± 0.42 ^c	3.66 ± 0.18 ^c
	50mg/kg b.w.t of extract + MP	72.98±1.16 ^d	34.48 ± 0.45 ^d	3.15 ± 0.06 ^{bc}
	50mg/kg b.w.t of extract without MP	78.44±0.91 ^{ef}	36.52 ± 0.92 ^e	2.87 ± 0.07 ^{ab}

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

5. Discussion

Abnormalities in liver function indices and in the activities of alanine transaminase, aspartate transaminase, alkaline phosphate, and the concentrations of total protein, total bilirubin, and albumin during malaria infection have been reported in literature [1, 4]. Changes in the activities of these enzymes and concentrations of the indices have been attributed to the extra-erythrocytic life cycle of *Plasmodium* spp that affects the liver. The present study focused on evaluating the effects of some phytochemicals

extracted from *Colanitida* leaf on liver function indices of mice infected with *Plasmodiumberghei* (NK-65). The antimalarial activity of *Cola nitida* leaf has been previously reported [9].

Findings in this study revealed that infection with the parasite caused abnormalities in the activities of the assayed enzymes (Table 1). This agrees with the findings of [5, 4] who also reported similar findings and stated that the derangement of these liver function enzymes activities is due to the hepatotoxic effects elicited by the products of erythrocyte

destruction by the parasites or membrane disruption activities of the parasites during their extra-erythrocytic life cycle in the liver. However, treatment of the infected mice with all the phytochemical extracts (at the doses of 25 and 50mg/kg b.w.t) was found to significantly improve the activities of the enzymes towards normal. This effect may be linked to membrane stabilization and maintenance of hepatocyte integrity potentials^[21] by preventing the leakage of liver enzymes (AST, ALT and ALP) into the circulatory system, antioxidant activity of these phytochemicals which reduces hepatic oxidative stress^[22, 9] and parasite clearance activity of the phytoconstituents^[9]. Therefore, these phytochemicals can be said to have been effective in restoring liver function indices distorted by malaria infection.

The reduction of total protein and albumin due to infection was also restored towards normal after treatment (Table 2). The observed increase in concentration in the case of bilirubin was also improved by treatment. Reduction in RBC destruction by *Plasmodium berghei* due to parasite clearance and antioxidant effect of these phytochemicals^[9] might be responsible for the reduction in serum concentration of bilirubin in the treated groups; since bilirubin is a derivative of RBC destruction^[23, 4].

6. Conclusion

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

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