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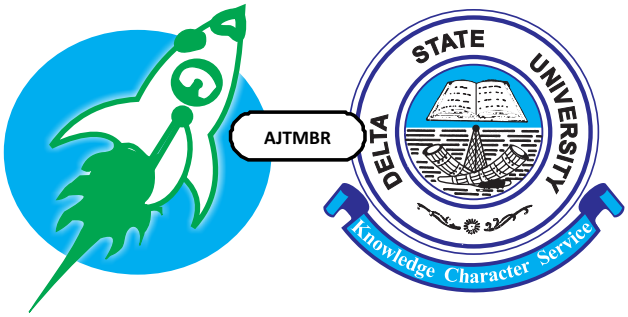

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Focus and Scope

The African Journal of Tropical Medicine and Biomedical Research is a multidisciplinary and international journal published by the College of Health Sciences, Delta State University of Abraka, Nigeria. It provides a forum for Authors working in Africa to share their research findings on all aspects of Tropical Medicine and Biomedical Sciences and to disseminate innovative, relevant and useful information on tropical medicine and biomedical sciences throughout the continent. The journal will publish original research articles, reviews, editorials, commentaries, short reports, case reports and letters to the editor. Articles are welcome in all branches of medicine and dentistry including basic sciences (Anatomy, Biochemistry, Physiology, Pharmacology, Psychology, Nursing etc) and clinical sciences (Internal Medicine, Surgery, Obstetrics and Gynaecology, Dental surgery, Child Health, Laboratory Sciences, Radiology, Community Medicine, etc). Articles are also welcome from social science researchers that document the intermediating and background social factors influencing health in countries of Africa. Priority will be given to publication of articles that describe the application of the principles of primary health care in the prevention and treatment of diseases.

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Original Articles

Phytochemical, Acute Toxicity, and Anti-plasmodial Potential of Concomitant Extracts of *Azadirachta indica* and *Mangifera indica* on Liver Function and Microscopic Anatomy in Swiss Mice

[1]Udoh, MI[1,2], Edagha IA, Peter AI[3], Udobang JA, [1]Peter AJ, [1]Udotong IU, [1]Ataben MA

Abstract

Introduction: In sub-Saharan Africa, malaria is the predominant contributor to mortality and there is significant dependence on phytotherapy for its treatment. This study investigated comparative phytoconstituents, acute toxicity, anti-plasmodial activities on liver function and microanatomical perturbations following the concomitant administration of ethanol extracts of *Azadirachta indica* leaves and *Mangifera indica* bark in *Plasmodium berghei*-infected Swiss mice.

Materials and Methods: Sixty experimental mice were allotted into 12 groups (n = 5) and inoculated with 1 x 10^6 *P. berghei* two weeks post- acclimatization. Group one served as the normal control; group two [parasitized-non-treated] and groups 3 to 11 were low, medium, and high doses of the extracts singly and concomitantly, while group 12 received artemether-lumefantrine. All administrations were via oral route for three days, respectively. Phytochemical screening, parasite density, serum liver enzymes and microanatomical alterations were analyzed.

Results: Phytochemistry showed that *A. indica* possessed abundant alkaloids that were absent in *M. indica*. The median lethal dose (LD50) of *A. indica* leaf and *M. indica* bark extract was 3240.37 and 2738.61 mg/kg, respectively. The single administration of *A. indica* outperformed *M. indica* via mitigated parasite progression, reduced *P. berghei*-induced hepatotoxicity and elevated liver enzymes.

Conclusion: *Azadirachta indica* surpasses *Mangifera indica* in alleviating hyperparasitemia, parasite-associated hepatotoxicity, and hepatic microanatomical changes in *in vivo* rodentia malaria model. *A. indica* also mild to moderately improved hepatic collagen and glycogen storage than *M. indica*. It possessed a better synergistic effect than *M. indica* alone.

Keywords: *Azadirachta indica*, *Mangifera indica, Plasmodium berghei*, anti-plasmodial, liver function

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INTRODUCTION

Malaria is an infectious disease that can be life-threatening and is spread by mosquitoes to humans and other animals. It is caused by the parasite protozoans that belong to the genus Plasmodium[1]. The disease is spread to people through the bites of infected female *Anopheles* mosquitoes. There are about four species of malaria parasites that can infect rodents namely, *Plasmodia berghei, vinckei, yoelli,* and *chabaudi,* respectively. Amongst these parasites, *Plasmodium berghei* is preferably used as model for experimental studies. It preferentially infects normocytes and reticulocytes and usually produces infections in mice that induce severe pathology[2].

Artemisua anua, is a Chinese plant whose major component forms the current gold-standard treatment for malaria is the artemisinin-based

combination therapy (ACT). It is worth noting that combination therapies, in which two or more antimalarial drugs with different mechanism of action are given together in fixed dose are quite effective for improved efficacy and reduced drug resistance by the parasites[3].

Several pharmacological studies have been reported in a recent review literature to demonstrate antimalarial plants either as crude extracts, or in purified metabolites for malaria treatment. Interestingly most of these plants show no evidence of parasite resistance because of their synergistic action of phytochemical components representing the great potential of the plant extract against malaria resistance[4]. Amongst several antimalarial plants used in folklore are; neem plant (*Azadirachta indica*) popularly called dongoyaro, and mango plant (*Mangifera indica*) found in many rural residences and now popular in tea mixture sold at malls and supermarkets based on preference.

Azadirachta indica is a tree in the Mahogany family Meliciceae[5,6]. It is native to tropical and semi-tropical climates and thrives in arid and savanna parts of Nigeria. Neem and its derivatives have been employed in ethnomedicine as antimalarials. The antimalarial effect of neem has been documented and is thought to act by redox perturbation in the form of imposition of substantial oxidant stress during malaria infection[7,8].

Mangifera indica is a tree from the *Anacardieceae* family that is widely found in tropical countries. This tree produces very sweet fruits. All the parts of a mango are very useful and thus a pharmacologically, ethnomedical, and phytochemically diverse plant[9]. The bark of a mango tree contains an active substance called mangiferin. It is known for its use in natural medicine, not only as a health enhancing panacea or adjunct therapeutic, but also for brain function improvement[10].

The liver is a key organ in the pathogenesis of malaria infection. It serves an important part of the life cycle of the parasite, being the organ where asexual reproduction (schizogony) of the mosquito infected sporozoites takes place[11,12]. Furthermore, certain clinical characteristics associated with severe malaria, including hypoglycemia, hyperparasitemia, jaundice, and bruising, are entirely or partially explained by liver damage caused by parasitemia or the immunological response to the parasite. This study investigated the phytochemical constituents, acute toxicity, and anti-plasmodial activity of the concomitant administration of *Azadirachta indica* and *Mangifera indica* extracts on the hepatic function and microanatomical alterations in Swiss Mice.

MATERIALS AND METHODS

The medicinal plant farm of the Department of Pharmacology and Natural Medicine, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria, provided fresh mature *Azadirachta indica* leaves and *Mangifera indica* bark. Both plants were washed to remove dirt and sun-dried for one week before being recognized and verified by taxonomists and placed in the Herbarium Unit of the Department of Botany, Faculty of Science, University of Uyo, Nigeria, with specimen voucher number (U.U.H. 63/19).

The dried plant materials were thereafter separately blended into a powdery form, and preserved separately in containers with appropriate covering, at room temperature. About 2.5 liters of absolute ethanol was used to mix 700 g of each powdered plant material and the mixture was kept for 72 hours at room temperature. They were filtered using linen and then filter paper. After 30 minutes of post-

filtration, the filtrates were evaporated to dryness using a water bath at 40 °C, and the extracts were stored in a sealed container in the freezer (2 – 8 °C) until it was used.

Extracts from both the *Azadirachta indica* leaf and bark of *Mangifera indica* were used for the phytochemical tests as described by Harborne[13], Sofowora[14] and Trease and Evans[15]. This procedure was done in the Pharmacology Department, Faculty of Pharmacy, University of Uyo, Nigeria.

This experiment involved sixty Swiss mice weighing between 20 and 30 g. The mice were sourced from the Animal House of the Faculty of Pharmacy at the University of Uyo in Akwa Ibom, Nigeria. The protocols for this study were approved by the Department of Human Anatomy, University of Uyo, Nigeria, which aligns with the globally accepted guideline for the use and handling of laboratory animals[15]. Additionally, an ethical approval was granted by the Health Research Ethics Committee of the Akwa Ibom State Ministry of Health with Ref: MH/PRS/99/Vol.IV/715. The study was conducted in accordance with the Basic and Clinical Pharmacology and Toxicology policy for experimental studies[16]. All animals were weighed, identified, and placed in a standard plastic cage for one week of acclimatization in an optimum pathogen-free environment, with a 12hour light/dark cycle of 25 – 27 °C and relative humidity of 40 - 60% measured using a CEM hydrometer (DT 615, Shenzhen China). The animal cages were maintained adequately by changing sawdust, leftover feed and drinking water, daily. All animals were fed with pelletized grower mash (Grand Cereal Vital® Feed Ltd. Jos) and provided drinking water *ad libitum*. The ARRIVE guidelines 2.0 as checklist of relevant information for animal research documentation of *in vivo* experiments was consulted.

Blood stage *Plasmodium berghei* samples were collected from a laboratory stock via serial blood passage from one mouse to another. This was sourced from the Faculty of Pharmacy at the University of Uyo.

Leaf extract of *Azadirachta indica* and bark extract of *Mangifera indica* were investigated to determine their medium lethal doses (LD_{50}) in the Department of Pharmacology, Faculty of Pharmacy, University of Uyo. This was done to establish the different amounts of the extracts to be administered to the mice using the method of Lorke[17].

Fifteen (15) animals were separated into five (5) groups of three (3) each. Each group received increasing doses of leaf/bark extracts (1000, 1500, 2000, 2500, and 3000 mg/kg). The animals were carefully monitored for 24 hours to assess their behavioural activities as well as the possibility of fatality. Where there was no mortality, another group of three (3) animals was given greater doses and monitored for 24 hours.

The inoculum was prepared as described by Edagha et al[18]. The blood donor with high parasitemia was acquired by anaesthetizing the mouse with ketamine hydrochloride and collecting blood via heart puncture with a sterile syringe into a sterile heparinized tube. The percentage parasitemia was calculated by dividing the number of parasitized red blood cells by the total number of red cells, and a desired volume of blood was obtained from the donor mouse and diluted with sterile normal saline so that the final inoculum (0.2 mL) for each mouse contained the required number of parasitized red blood cells (1.0×10^6 parasitized red blood cells), which is the standard inoculum for infection of a single mouse.

Infection of Swiss Mice with *P. berghei* and

administration of the Extracts of *Azadirachta indica* **and** *Magnifera indica*

The sixty Swiss mice were allotted into twelve (12) groups (n = 5) mice each. All the mice were infected two weeks after acclimatization with 1 x 10^6 *P. berghei* parasitized erythrocytes intraperitoneally, except the healthy control group that is, the normal control (NC). The infection of the recipient mice was initiated by needle passage of the inoculum parasite preparation from the donor to healthy animals via an intraperitoneal route as described by Edagha *et al*., (2014). The presence of parasites was confirmed by a daily parasitemia determination using direct enumeration via a Giemsa-stained thin blood smear from the mice's lateral caudal vein, which demonstrated the number of PRBCs per 100 RBCs of parasitemia as outlined by WHO in malaria microscopy in WHO[19]. The experimental groups commenced treatment when parasitemia reached 5% of the initial inoculum. The parasite density was calculated using the formula below:

$$\% \text{ parasitemia} = \frac{Number\ of\ parasitized\ RBC}{Total\ number\ of\ RBC} \text{ X } 100$$

Also the percentage inhibition of the parasite for each day was calculated by the formula:

$$\frac{Mean\ \%\ parasitemia\ of\ untreated\ group - Mean\ \%\ parasitemia\ of\ treated\ group}{Mean\ \%\ parasitemia\ of\ untreated\ group} \text{ X}100$$

Experimental Design

S/N	Group	Treatment	Duration (days)
1.	NC	DW 5 mL/kg bwt	3
2.	PNT	DW 5 mL/kg bwt	3
3.	PAI_{LD}	324 mg AI/kg bwt	3
4.	PAI_{MD}	648 mg AI/kg bwt	3
5.	PAI_{HD}	972 mg AI/kg bwt	3
6.	PMI_{LD}	274 mg MI/kg bwt	3
7.	PMI_{MD}	548 mg MI/kg bwt	3
8.	PMI_{HD}	822 mg MI/kg bwt	3
9.	$PAIMI_{LD}$	324 mg AI + 274 mg MI /kg bwt	3
10.	$PAIMI_{MD}$	648 mg AI + 548 mg MI /kg bwt	3
11.	$PAIMI_{HD}$	972 mg AI + 822 mg MI /kg bwt	3
12.	PAL	8 mg/kg bwt	3

DW – Distilled water, NC – Normal control, PNT – Parasitized non-treated, PAI_{LD} – Parasitized and treated with *Azadirachta indica* extract – low dose, PAI_{MD} – Parasitized and treated with *Azadirachta indica* extract – middle dose, PAI_{HD} – Parasitized and treated with *Azadirachta indica* extract – high dose, PMI_{LD} – Parasitized and treated with *Mangifera indica* – low dose, PMI_{MD} – Parasitized and treated with *Mangifera indica* – middle dose, PMI_{HD} – Parasitized and treated with *Mangifera indica* – high dose, $PAIMI_{LD}$ –

Parasitized and treated with *Azadirachta indica* extract and *Magnifera indica* extract – low dose, $PAIMI_{MD}$ – Parasitized and treated with *Azadirachta indica* extract and *Magnifera indica* extract– middle dose, $PAIMI_{HD}$ – Parasitized and treated *Azadirachta indica* extract and *Magnifera indica* extract– high dose, PAL – Parasitized and treated with Arthemeter Lumefantrine (ACT). All extracts treatment was administered orally using oro-gavage tube every day for 3 days.

Termination of the Experiment

Twenty-four hours following the last dose, the animals were anesthetized intraperitoneally with 50 mg per kg body weight of ketamine hydrochloride (Rotex Medica, Germany). The thoracoabdominal wall was dissected to examine the heart, and blood was taken from the left ventricle. The heparinized blood was collected immediately and centrifuged at 3000 g for 15 minutes. The plasma was refrigerated and processed as a single batch for determination of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) levels within 12 hours[20]. A cannula was utilized to administer a guided intracardiac perfusion of phosphate-buffered saline (PBS, 2M. PH 7.0) to the animals, which were then perfused-fixed with 10% buffered formalin. The livers of all animals were extracted and fixed in 10% buffered formalin for 48 hours.

Histopathological Assessment

The fixed tissue was dehydrated using graded alcohol to eliminate excess water found in tissues, as follows: two changes of 70% and 90% alcohol for two hours each, and two changes of 100% alcohol for two hours each. After dehydration, tissue was cleared with two changes of xylene (5 minutes each). This was followed by impregnation with two changes of paraffin wax in the oven at 60°C for one hour and thirty minutes each to allow for infiltration. Tissue was put into molds filled with molten paraffin wax. Once the paraffin wax had cooled and set, the mold was removed, revealing a small paraffin block containing the tissue sample. After freezing the tissues on ice, the paraffin blocks were sectioned with a microtome at a thickness of 5 μm.

Tissue sections were taken to water by deparaffinizing in two changes of xylene for 2 minutes each, followed by rehydration in 100% alcohol, 95% alcohol, 70% alcohol twice for 5 minutes each, and finally rinsed in tap water. Sections were stained with Hematoxylin for 10 minutes, washed briefly in tap water, differentiated in acid alcohol, washed well in running tap water till it turned blue. The blued sections were counterstained in Eosin solution for 1 minute[21]. The tissues were air dried and mounted with dipolycysteine xylene (DPX) before being cover-slipped for viewing under a light microscope. Three independent histopathologists unrelated to the study evaluated the tissues to mitigate study bias. The processed slides were examined under a light microscope (Olympus, USA), and photomicrographs were taken.

Statistical Analysis

The data obtained from this study was analyzed using the IBM Statistical Package for Social Science (SPSS) version 25 software, with the "One-Way" analysis of variance (ANOVA) and the Tukey post-hoc test to establish significance. Data was presented as mean ± Standard Error of Mean. Values were statistically significant ($p < 0.05$).

RESULTS

Phytochemical Analysis of Extracts of *Azadirachta indica* Leaf and *Mangifera indica* Bark

The different phytochemical constituents present in *Azadirachta indica* leaf extract and *Mangifera indica* bark extract are shown in Table 1.

The qualitative phytochemical analysis of the

extract of *Azadirachta indica* revealed the presence of alkaloids, tannins, flavonoids, polyphenols and terpenoids in high concentration, saponins, carbohydrate, glycoside, and cardiac glycoside in moderate concentrations, while steroid was present in trace concentration. On the other hand, the phytochemical constituents of extract of *Mangifera indica* were tannins, flavonoids, polyphenols, and glycosides, all present in high concentrations; saponins and carbohydrate were present in moderate concentrations; cardiac glycosides, steroids and terpenoids were also in trace concentration while alkaloid was absent.

Median Lethal Dose (LD_{50}) of Extracts of *A. indica* **Leaf and** *M. indica* **Bark**

There was no toxicity or mortality seen as the dose of *Azadirachta indica* leaf extracts increased to 3000 mg/kg body weight. The median fatal dose of *Azadirachta indica* extract was found to be more than 3000 mg/kg, (Table 2a).

The ethanolic extract of *Mangifera indica* bark revealed no toxicity or death at doses as high as 2500 mg/kg body weight. The median fatal dose was predicted to be more than 2500 mg/kg body weight, (Table 2b).

Effect of Extracts of *A. indica* **Leaf and** *M. indica* **Bark on Body and Liver Weights of** *P. berghei*-**infected Swiss Mice**

The post passage weight showed a significant ($p < 0.05$) reduction of the weights in groups 4, 5, 7, and 10 respectively, when compared to the NC. Although, group 9 was significantly ($p < 0.05$) increased in weight when compared to the groups 4 and 7, group 8 also was significantly ($p < 0.05$) increased when compared to the groups 4, 5, and 7.

Final body weight demonstrated a significant ($p < 0.05$) reduction of weight in groups 2, 3, 4, 5, 7, 9, and 10 respectively when compared to the control group. Although, observations showed a significant ($p < 0.05$) decrease in the groups 7 and 9, there is a significant ($p < 0.05$) increase in the groups 6, 8, 11 and 12 when compared to the diseased group (group 2).

Analysis also observed that there were negative changes in weight in all the treatment groups except in group 12, which showed a positive change in weight when compared to the control and other treatment groups, as shown in Table 3.

Liver weight analysis demonstrated no significant ($p > 0.05$) differences across treatment groups when compared to the control group, as shown in Table 3.

Effect of Extracts of *A. indica* **Leaf and** *M. indica* **Bark on Serum Liver Enzymes of** *P. berghei*-**infected Swiss Mice**

The serum AST concentration showed a significant ($p < 0.05$) increase in groups 2 and 6 when compared to the control group. There was a significant ($p < 0.05$) decrease in AST concentration of group 12 when compared to the parasitized non-treated (PNT) group.

Assessment of the ALT presented a significant ($p < 0.05$) increase in ALT concentration across all treatment groups except for groups 4, 5, and 12 when compared to the control group. Whereas, groups 3, 4, 5, 7, 8, 10, 11 and 12 showed a significant ($p < 0.05$) decrease in ALT concentration when compared to the PNT group, group 5 and 12 showed a significant ($p < 0.05$) reduction when compared to group 9 and a significant ($p < 0.05$) reduction in groups 5 and 12 when compared to the group 7.

Result presentation showed a significant ($p < 0.05$) elevated ALP concentration in the groups 2 and 6 when compared to the control group. However, all other treatment groups showed a significant ($p < 0.05$) reduction in ALP concentration when compared to the PNT group (group 2), as shown in Table 4.

Effect of Extracts of *A. indica* **Leaf and** *M.*

***indica* Bark on Parasitemia of *P. berghei*-infected Swiss Mice**

The assessment of initial parasitemia level across all treatment groups showed no significant ($p > 0.05$) difference when compared to the PNT group (group 2), as presented in Table 5. Final parasitemia showed a significant ($p < 0.05$) decrease in all the treatment groups when compared to the PNT group (group 2). However, a more significant ($p < 0.05$) decrease is observed in the groups 11 and 12 when compared to other treatment groups as well as the PNT group (group 2).

Effect of Extracts of *A. indica* Leaf and *M. indica* Bark on the Histology of the Liver of *P. berghei*-infected Swiss Mice

The section of the liver of group 1 (NC) showed a regular histoarchitecture with normal array of hepatocytes (HP) and portal triad as shown in Figure 1. The liver section of group 2 animals (PNT) revealed severely distorted histoarchitecture demonstrating the presence of degenerating hepatocytes (D), numerous hepatic vacuolations (V), widespread microvascular steatosis (St) and widely populated parasites (p). Groups 3 to 11 demonstrated mild to moderate reversal of liver distortions when compared to NC, and group 12 treated with standard anti-malarial drug (PAL).

Collagen expression was demonstrated in Figure 2, showing intense Masson's trichrome uptake in the *Mangifera indica* bark extract treatment groups particularly medium dose (group 7), the concomitant extract groups exhibited moderate expressions (groups 9 to 11) as well as groups 1, 3, 4, and 5, the low expression groups were PNT and particularly the PAL group.

Glucose deposition demonstrated by periodic acid Schiff was most expressed in groups NC and PAL. Other test groups expressed low to slightly moderate glucose storage, as presented in Figure 3.

Table 1: Phytochemical Screening for the Ethanolic Leaf Extract of *Azadirachta indica* and Bark Extract of *Mangifera indica*

S/N	Phytochemical Constituents	*Azadirachta indica*	*Mangifera indica*
1.	Alkaloids: (a). Dragendoff Reagent	+++	-
	(b). Mayer's Reagent	+++	-
2.	Saponin	++	++
3.	Tannin	+++	+++
4.	Carbohydrate	++	++
5.	Flavanoid	+++	+++
6.	Polyphenol	+++	+++
7.	Cardiac Glycoside	++	+
8.	Steroids	+	+
9.	Terpenoids	+++	+
10.	Glycoside	++	+++

+ = present (+ = trace; ++ = moderate concentration; +++ = high concentration); - = absent

Table 2a: Median Lethal Dose for the Ethanolic Leaf Extract of *Azadiractha indica*

Group (n = 3)	Dose of *Azadirachta indica* extract (mg/kg)	Mice Mortality
1.	1000	None
2.	2000	None
3.	3000	None
4.	3500	1

$LD_{50} = \sqrt{AB}$; Where A = The maximum dosage that produce 0 % mortality and
B = The minimum dosage that produces 100 % mortality.

A. *indica* = $\sqrt{3000X3500}$ = $\sqrt{10,500,000}$ = 3240.37 mg/kg

10 %, 20 % and 30 % of the value was used for the main experiment

Table 2b: Median Lethal Dose for the Ethanolic Extract of *Mangifera indica* Bark

Group (n = 3)	Dose of *Mangifera indica* extract (mg/kg)	Mice Mortality
1.	1000	None
2.	2000	None
3.	2500	None
4.	3000	1

LD_{50} is over 2500 mg/kg body weight of *Mangifera indica.*

Likewise using the above formula *M. indica* = $\sqrt{2500X3000}$ = $\sqrt{7,500.000}$ = 2738.61 mg/kg.

10 %, 20 % and 30 % of the value was used for the main experiment

Table 3: Effect of Extracts of *A. indica* Leaf and *M. indica* Bark on Body Weight of *P. berghei*-infected Swiss Mice

S/N	Group	Initial BW (g)	Post Passage W. (g)	Final BW (g)	Change in W (g)	% Change in Weight	Liver Weight (g)
1.	NC	25.74 ± 0.75	26.56 ± 0.56[a]	25.65 ± 0.53	0.09	0.35	1.41± 0.07
2.	PNT	23.53 ± 1.33	23.85 ± 1.44	17.65 ± 3.97	- 5.88	33.31	1.66± 0.13
3.	PAI_{LD}	24.16 ± 0.99	26.40 ± 0.86[a]	20.83 ± 3.06	- 3.33	15.98	1.64± 0.12
4.	PAI_{MD}	21.60 ± 0.68	20.43 ± 2.98	18.50 ± 2.70	- 3.10	16.70	1.47± 0.14
5.	PAI_{HD}	22.56 ± 0.64	21.48 ± 3.15	16.88 ± 3.71	- 5.68	33.61	1.75± 0.33
6.	PMI_{LD}	25.70 ± 0.89	26.63 ± 1.19[a]	25.85 ± 1.00	- 0.19	0.58	1.61± 0.12
7.	PMI_{MD}	21.33 ± 0.66	19.80 ± 2.99	14.85 ± 3.30	- 6.48	43.63	1.36± 0.14
8.	PMI_{HD}	30.45 ± 1.31	30.13 ± 1.07[a]	24.43 ± 3.55	- 6.02	24.59	1.89± 0.03
9.	$PAIMI_{LD}$	27.09 ± 1.22	29.51 ± 1.64[a]	23.09 ± 3.19	- 3.52	16.82	1.78± 0.00
10.	$PAIMI_{MD}$	22.33 ± 1.14	22.19 ± 1.01	17.99 ± 2.68	- 4.34	24.12	1.58± 0.20
11.	$PAIMI_{HD}$	23.69 ± 0.73	25.03 ± 0.99	23.16 ± 0.77	- 0.53	2.28	1.61± 0.08
12.	PAL	23.35 ± 0.97	25.13 ± 0.52	24.33 ± 0.68	0.98	4.07	1.39± 0.24
	P value	-	0.0005	-	-	-	0.471

Values are expressed as Mean ± SEM; n = 5; a = $p < 0.05$ relative to PMI_{HD}.

Table 4: Effect of Extracts of *A. indica* Leaf and *M. indica* Bark on Serum Liver Enzymes of *P. berghei*-infected Swiss Mice

S/N	Group	AST (U/L)	ALT (U/L)	ALP (U/L)
1.	NC	146.7 ± 2.59	57.76 ± 2.59	52.86 ± 2.70
2.	PNT	169.1 ± 2.32[a]	84.75 ± 2.68[a]	75.6 ± 2.38[a]
3.	PAI_{LD}	157.8 ± 6.76	72.71 ± 3.08[a,b]	61.87 ± 5.10
4.	PAI_{MD}	154.1 ± 2.47	66.41 ± 2.57[b]	60.11 ± 1.34
5.	PAI_{HD}	153.1 ± 1.31	64.09 ± 2.27	59.09 ± 0.74
6.	PMI_{LD}	167.1 ± 2.20[a]	78.20 ± 1.38[a,b,c]	65.11 ± 1.46[a]
7.	PMI_{MD}	163.9 ± 3.33	70.13 ± 0.85[a,b]	58.64 ± 0.77
8.	PMI_{HD}	163.8 ± 2.50	68.44 ± 1.28[a,b]	57.16 ± 1.88
9.	$PAIMI_{LD}$	155.2 ± 2.11	75.08 ± 2.58[b]	60.56 ± 2.00
10.	$PAIMI_{MD}$	155.8 ± 8.04	73.54 ± 0.80[a,b]	59.04 ± 2.40
11.	$PAIMI_{HD}$	151.9 ± 4.31	70.61 ± 1.69[a,b]	56.47 ± 1.59
12.	PAL	148.1 ± 2.75	64.91 ± 1.07	56.20 ± 2.08
	P value	0.0027	0.0001	0.0001

Values are expressed as Mean ± SEM; n = 5; a = $p < 0.05$ relative to NC; b = $p < 0.05$ relative to PNT; c = $p < 0.05$ relative to PAI_{HD} and PAL.

Table 5: Effect of *A. indica* Leaf Extract and *M. indica* Bark Extract on Parasitemia of *P. berghei*-infected Swiss Mice

S/N	Group	% Initial Parasitemia	% Final Parasitemia
1	NC	0.00 ± 0.00	0.00 ± 0.00
2	PNT	48.00 ± 2.08	53.00 ± 1.95[a,b]
3	PAI_{LD}	47.75 ± 1.54	36.00 ± 0.91[a,b]
4	PAI_{MD}	48.00 ± 1.95	31.50 ± 1.55[a,b]
5	PAI_{HD}	47.75 ± 1.37	27.50 ± 1.25[a,b]
6	PMI_{LD}	47.75 ± 2.59	36.75 ± 1.75[a,b]
7	PMI_{MD}	48.50 ± 1.84	33.50 ± 1.70[a,b]
8	PMI_{HD}	48.75 ± 3.63	29.50 ± 2.10[a,b]
9	$PAIMI_{LD}$	48.50 ± 2.32	32.25 ± 3.35[a,b]
10	$PAIMI_{MD}$	48.75 ± 3.17	29.75 ± 2.86[a,b]
11	$PAIMI_{HD}$	48.75 ± 2.17	15.75 ± 2.62[a]
12	PAL	50.00 ± 1.08	8.50 ± 0.64[a]
	P value	-	0.0001

Values are expressed as Mean ± SEM; n = 5; a = $p < 0.05$ relative to NC; b = $p < 0.05$ relative to $PAIMI_{HD}$ and PAL.

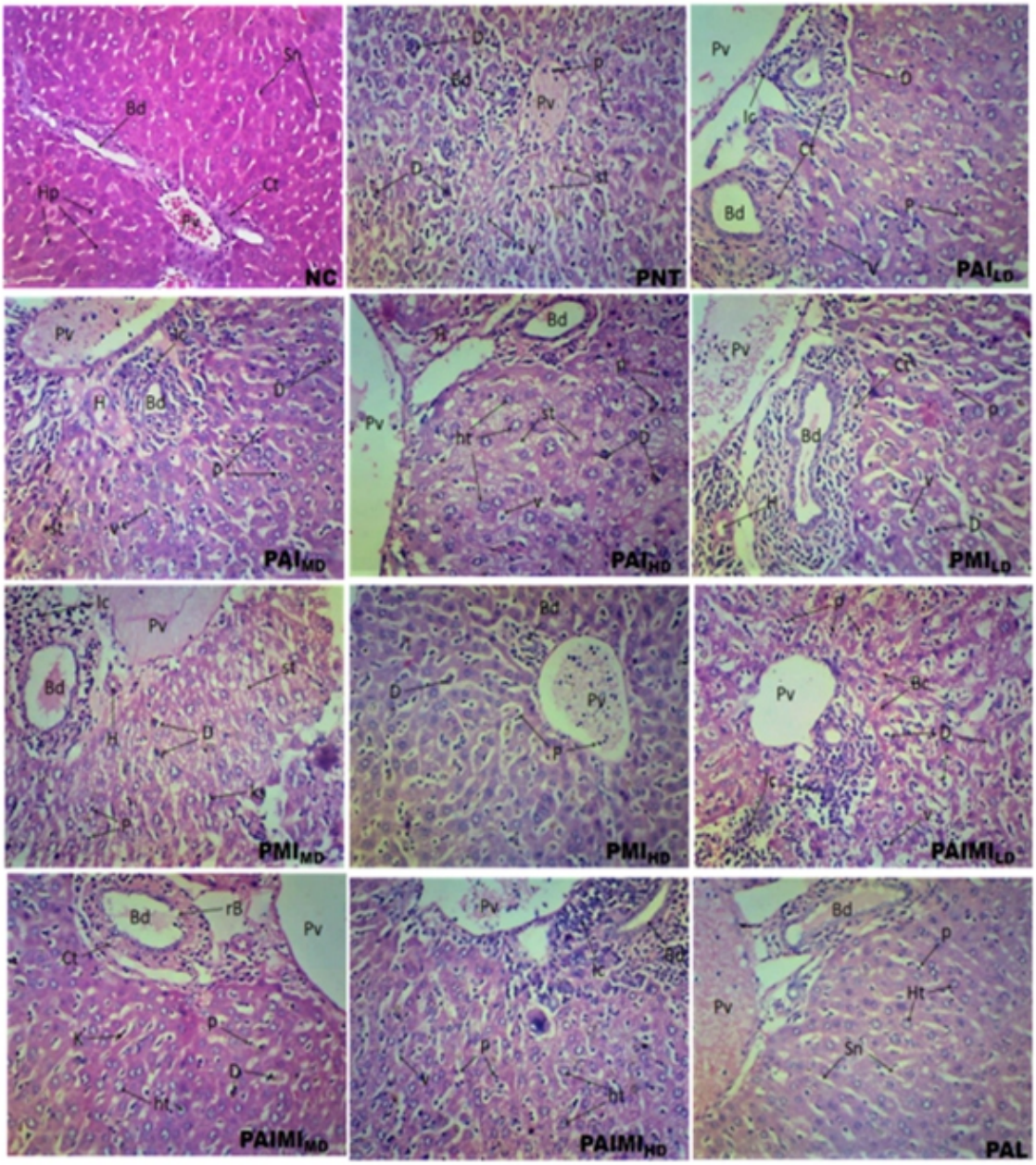

Figure 1: Representative photomicrographs of the cross section of the liver showing normal control (NC), that is group 1 and test groups 2 to 12 respectively. H&E x400. Legend: **S**inusoidal (Sn) arrays within the hepatic lobules, populated hepatocytes (Hp), Portal vein (Pv), Bile duct (Bd), and connective tissue cells (Ct) within the portal area.

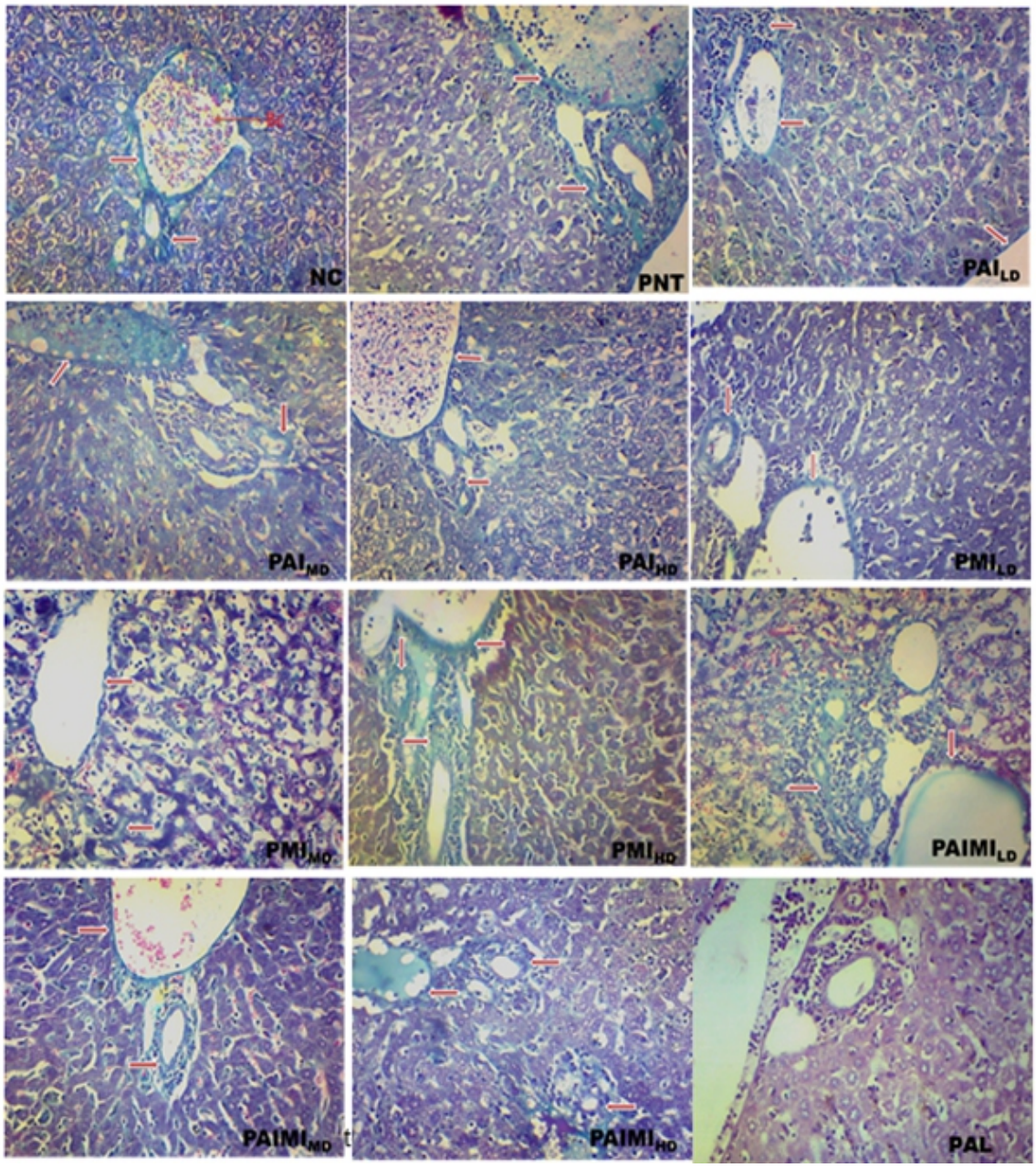

Figure 2: Representative photomicrographs of the cross section of the liver showing normal control (NC), that is group 1 and test groups 2 to 12 respectively. Masson's trichrome x400. Legend: Red arrow = collagen expression.

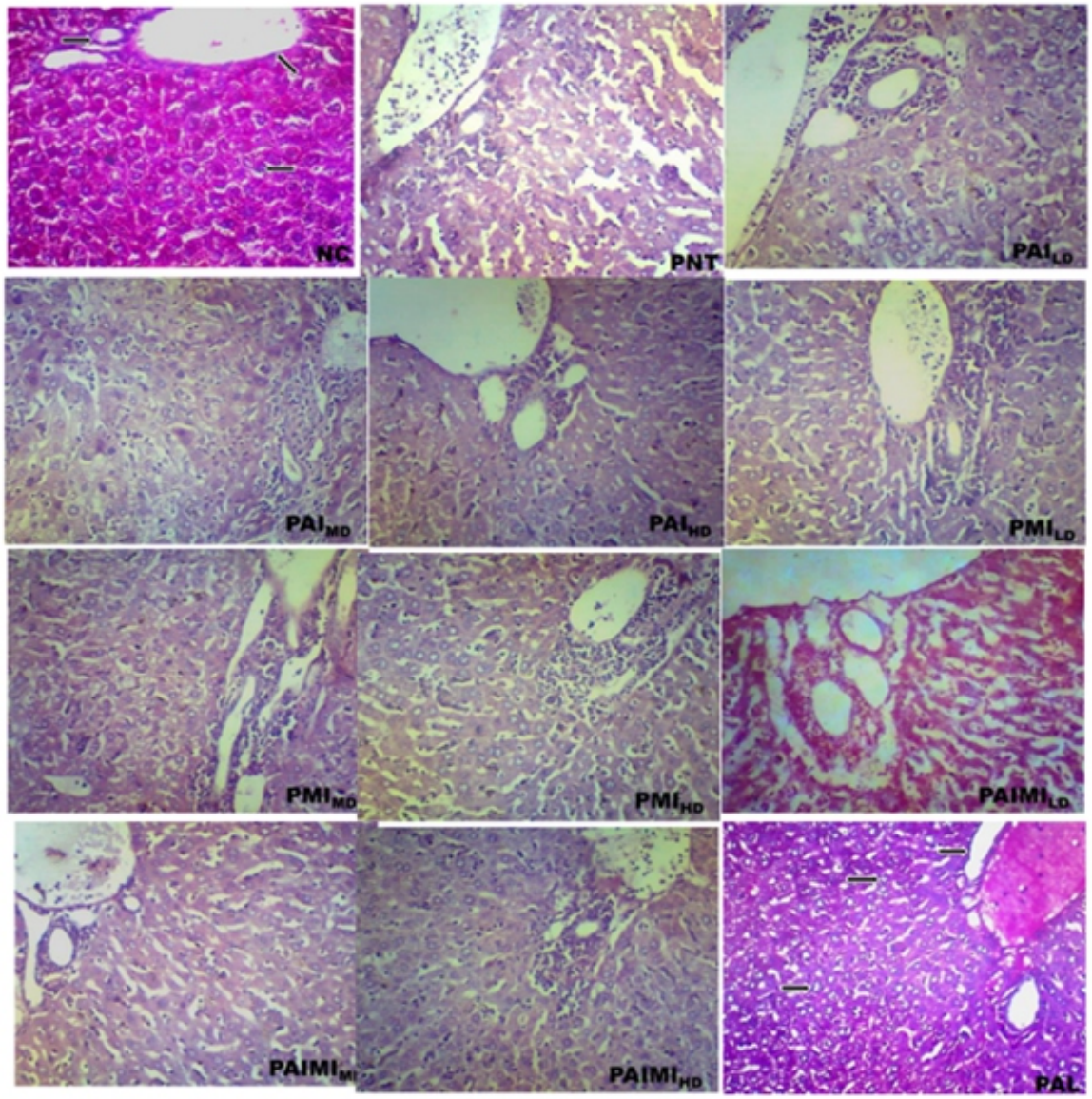

Figure 3: Representative photomicrographs of the cross section of the liver showing normal control (NC), and the test groups, respectively. Periodic acid Schiff, x400. Legends: NC: showing normal glycogen expression. Parasitized groups demonstrated depleted glycogen expression except in PAIMI$_{LD}$ and PAL which had up-regulated glycogen contents.

DISCUSSION

Herbal tea mixtures are now a popular trend, and many contain two or more blends of plants, mostly consumed for their health benefits. Here we investigated the concurrent administration of two popular anti-malarial plants; ethanol leaf extract of *Azadirachta indica* and *Mangifera indica* bark extract.

The phytochemical screening of the extracts

revealed that both extracts were rich in saponin, tannin, carbohydrates, flavonoid, polyphenol and glycoside and there was moderate concentration of steroids. *A. indica* was also rich in alkaloids but no trace of alkaloids was found in *M. indica,* also, *A. indica* contains more cardiac glycosides, and terpenoids than *M. indica.* The phytochemicals present in these extracts may support their medicinal efficacy and established pathways of action against many diseases. Several of these phytochemicals have been shown to have anti-plasmodial effects. Alkaloids are reported to block parasitic protein synthesis[22,23]. In this study, parasitized mice treated with *A. indica* showed impeded parasite growth compared with PNT group. Flavonoids are one of the most important phytochemicals detected in both extracts. Flavonoids are capable of impairing *Plasmodium* nucleic acid base pairing[23]. Saponins have antimalarial, cytotoxic, and anti-tumor activities[23,25] and plants containing saponins have been used for medicinal purposes for decades[26]. Saponins, tannins and phenols also pose some anti-plasmodial activity. *A. indica* possesses a higher concentration of terpenoids and terpenoid possesses effects detrimental to infective protozoans, including *Plasmodium*[27,28]. The phytochemicals screening also showed that the extracts of *A. indica* had alkaloids which were absent in *M. indica,* and more terpenoids. This could indicate that the *A. indica* extract has greater biological activity.

Acute toxicity study on the ethanolic leaf extracts of *Azadirachta indica* showed that the median lethal dose of the extract is over 3000 mg/kg body weight, this value is like those obtained by Achi et al[28]. While the median lethal dose of the ethanolic extract of *Mangifera indica* bark was calculated to be over 2500 mg/kg body weight, this value is like those obtained by Reddeman et al[29]. These values demonstrate that *A. indica* has lower acute toxicity than *M. indica.* According to Tabuti et al[30] both plants are potent antimalaria plants and are slightly toxic, although safe for human consumption.

Conventionally, body weight gains often result from physiological variation such as food intake, and metabolism[31]. Therefore, changes in body weight can thus be used to examine individual response to pharmacological effects (both herbal and conventional). Also, it may indicate the side effects of drug[32]. As earlier reported by Farah et al[33] significant changes in body and internal organ weights are considered sensitive indices of toxicity after exposure to toxic substances. A gradual loss of appetite and weight are usually seen in most if not all clinical manifestations of established malaria infection. Thus, the change in body weights of the experimental individuals indicated that malaria parasitemia was associated with considerable weight loss. The observed weight loss in the PNT is most likely owing to the negative consequences of malaria parasitemia. Treatment with Artemether/Lumefantrine - Coartem® (group 12) was associated with a significant weight gain. This agrees with the findings of Uraku[34] and Ozoko et al[35]. The rebound rise in body weight after treatment with Coartem® (ACT) is most likely attributable to a reversal of the negative effects of malaria parasitemia in mice. This recovery is interesting especially when compared to the findings by Samuel et al[36] who reported that administration of ACT or artesunate in unparasitized rats resulted in significant weight loss. It looks odd that untreated malaria parasitemia would be associated with weight loss, whereas parasitized treated with ACT was still associated with weight loss. However, the persistent weight loss observed in the treatment groups that received ethanol extracts of *M. indica* and *A. indica,* may be because of the presence of saponins and tannins (due to bitterness and astringent properties of

these respective phytochemicals), as previously reported by Yemitan and Adeyemi[37].

This study indicates that *A. indica* had better anti-plasmodial effect compared to *M. indica* when administered separately. However, there is a remarkable anti-plasmodial effect following the combination of both *A. indica* and *M. indica* on the parasite. This may be because of the chemical interaction between the phytochemical constituents of both plants' extracts, as well as their synergistic anti-plasmodial effect. This corroborates the conclusion drawn by Ofori-Attah et al[38] that reported the use of these concoctions in folkloric medical settings in the treatment of malaria. A combination of both single plant extract increased the concentration of polyphenols, thus improving their anti-plasmodic activities. Polyphenols elevate red blood cell oxidation and inhibit the parasites protein synthesis[39]. This activity nullifies the oxidative damage induced by the malaria parasite[40]. Thus, the strong antimalarial activity observed may be due to the presence of numerous polyphenols.

The commonest enzymes regarded as indicators of liver damage are aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT). The damage to hepatocellular cells results in the increase in these enzymes[41]. This research showed that there is a significant ($p < 0.05$) increase in AST level especially in the group with PNT animals as compared to the normal control (NC). However, extracts caused significant reduction in AST, ALT, and ALP activities in treated groups.

This research work has demonstrated the effect of aqueous extract of *A. indica* and *M. indica* on the liver. This claim has been supported by Prasad et al[42] who reported on the hepatoprotective potentials of these herbal compounds. Histopathological assessment using H&E technique was employed to study the hepatic tissue alterations induced by material parasites. The PNT group showed varying levels of hepatotoxic injuries and degenerations of hepatocytes, cellular vacuolations, and widespread micro-vesicular steatosis and evidence parasites present in the blood vessels and within sinusoids of the hepatic lobules. Result also showed varying levels of this inflammatory and degenerative condition in the extract administered. Liver involvement in severe malarial infection is commonly a significant cause of mortality among humans[43]. It was also documented in the study that hepatopathological changes of severe malarial parasite cases were associated with total bilirubin levels, apoptosis of Kupffer cells and portal tracts lymphocyte activation[43]. It is therefore an established fact based on findings that *Plasmodium* strains are localized in the liver of the first phase of the infection causing varying levels of hepatotoxic effects[44], as shown in this study.

A. indica and *M. indica* exhibit hepatoprotective activities[45,46]. Although the hepatic pathological features observed in the PNT group were also obvious in some of the extract treated groups, both in the single and in combination groups, the intensity of these features were mild, and reversed in the standard drugs treatment group when compared to NC group. The beneficial and toxic activities of tannins have also been investigated and documented[47]. The presence of tannins in both extracts of study may be implicated in the residual hepatotoxic changes observed in the result.

Furthermore, as revealed by the periodic acid Schiff staining, glycogen depletion in the liver was observed in all treatment groups besides the normal control and positive control (ACT-treated). Glycogen is a stored form of glucose, the

primary source of human energy received from carbohydrates absorbed through meals. The skeletal muscles and liver are the two main storage facilities for glycogen. Due to its pivotal role in metabolic fuel production, depletion of glycogen in the liver, the metabolism hub of the body could be linked to reduction in body weight as observed in the *M. indica* and *A. indica* extract-treated groups. Though the mechanism by which the extracts may likely induce glycogen depletion in the experimental groups remains unclear, following this storage depletion, there may be a probable cellular competition for available glucose after food intake. Therefore, insufficient glucose or a lack thereof may hinder several cellular activities including repairs and regeneration, which will negatively impact on the overall body weights of the experimental animals. This is unison with the report of Lacombe et al[48] who reported that after glycogen-depleting exercise, significant decreased in body weights were recorded before incremental exercise test in a horse model.

Glycogen storage has been a documented function of the liver[49]. The presence of these stored molecules in the liver has been studied applying the periodic acid Schiff technique[50]. As a histochemical technique, it is utilized to assist in the examination of tissues. The liver section stained for glycogen will display magenta staining, but with the PAS samples (depleted sample cells) will have a profound loss/absence of the magenta staining[51]. This study demonstrated a high expression of glycogen stain in the liver tissue of the normal control group and the standard drug (AL) treated group. The profound absence of glycogen expression in the PNT group and the extract treatment groups may be due to competitive activities of the parasites for systemic glucose to be utilized and stored by the hepatocytes, which is distinct from the presence of glycogen in the hepatic tissue of the AL (standard drug) treated group may be due to the drug toxicities to the parasites, causing their destruction hence lessening the competitive tendencies of the parasites for liver glucose, and hindering of glycogen storage within tissue.

CONCLUSION

Alkaloids are abundantly present (+++) in *Azadirachta indica* leaf extract but totally absent (-) in *Mangifera indica* bark extract. Notably, the median lethal dose (LD_{50}) demonstrated that *Azadirachta indica* (3240.37 mgkg) comapred to *Mangifera indica* (2738.61 mg/kg). Interestingly, *A. indica* single doses outperformed *M. indica* in reversing *P. berghei*-induced hepatotoxicity through mitigating parasite progression and associated elevated liver enzymes. Dose-dependent concentrations of *M. indica* severely altered hepatic collagen and glycogen storage compared to *A. indica.*

Author Contributions

MIU, IAE, AIP, and JAU conceptualized and designed and supervised the study, AJP, IUU and MAA performed the experiments, analyzed the data, and co-drafted the manuscript with MIU, IAE, AIP, and JAU.

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Conflicts of Interest

There are no conflicts of interest.

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