

Full Length Research Paper

Evaluation of herbal cocktail used in the treatment of malaria on liver tissue of adult Wistar rats

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Received 17 August, 2018; Accepted 17 September, 2018

Interest is renewed in herbal medicine since it is believed it has less side effects and is safer. In addition, there has been continued demand to obtain more drugs from plant sources to alleviate various ailments of mankind. This study is aimed at investigating the antimalarial activity in an herbal cocktail and individual plant extracts contained on *Plasmodium berghei* in infected Wistar rats. Thirty five Wistar rats randomly assigned into seven groups of five were used. The cocktail and individual aqueous extracts of *Azadirachta indica*, *Mangifera indica*, *Carica papaya*, and *Citrus limon* were orally administered to the infected Wistar rats weighing an average of 200 g at standard doses of 100 mg/kg/day for seven days, with the exception of aqueous leaf extract of *A. indica* which was administered at a dose of 10 mg/kg/day. The therapeutic effects of the cocktail and the individual extracts against *P. berghei* were investigated and the effects on the liver were histologically assessed. Biochemical assays for liver markers aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP) and albumin (ALB) were also assessed. The results showed that the cocktail and individual extracts possess antimalarial activity, by reducing the degree of parasitaemia, inducing recovery of hepatic cells and reduction of malaria associated liver pathology. Administration of the extracts did not significantly alter the level of albumin and total protein, no increase was observed in AST activity ($P > 0.05$). Significant increase was observed in the ALT activities among the rats administered with the cocktail or that which contained extract ($P < 0.0001$). In conclusion, the cocktail and the individual extracts possess antimalarial activity, thus justifying their usage in traditional medical practice. Extensive studies for validation of various medicinal plants used in treating malaria should be further conducted

Key words: *Plasmodium berghei*, cocktail, aspartate aminotransferase, alanine aminotransferase, total protein, albumin.

INTRODUCTION

Phytotherapy has been a backbone of medicine since way back in which various herbs and their extracts containing active ingredients of therapeutic significance is used (Tiwari et al., 2014). Natural plant extracts contain a variety of phenolic compounds which are assigned

various biological activities (Makni et al., 2018). Malaria remains a public health problem worldwide and a leading cause of death and disease in many developing countries especially the tropical and subtropical regions of the world where young children and pregnant women are

mostly affected (Vineet and Bagai, 2014; Iyama and Idu, 2015).

Malaria is one of the most common vector-borne diseases recognized as a crucial parasitic disease of humans, it is a life-threatening blood disease caused by parasites transmitted to human through female anopheles mosquitoes bite (Arzoo and Kumari, 2017). Until now, malaria is still a health problem worldwide, especially in tropical countries like Africa and Asia and about 3.3 billion people worldwide are at risk of malaria (Taek et al., 2018). Variety of Nigerian medicinal plants has demonstrated antimalarial potential and could serve as possible sources of antiplasmodial compound (Ibrahim et al., 2012). The World Health Organization defines herbal medicines to include herbs, herbal materials, herbal preparations and finished herbal products; containing active ingredients plants parts, or other plant materials, or combinations of both (WHO, 2005). In Nigeria, malaria remains a major public health problem and the high cost of the effective antimalarial drugs, poor quality drugs and increased emergence of Plasmodial resistance necessitates the need for alternative source of medicine in malaria treatment and prevention (Okere et al., 2014). Malaria is responsible for approximately 60% of outpatient visits and 30% of admissions in Nigeria and it is also believed to contribute up to 11% of maternal mortality, 25% of infant mortality, and 30% of under-5 mortality (FMOH, 2015). It is estimated that about 110 million clinically diagnosed cases of malaria and nearly 300,000 malaria-related childhood deaths occur each year (FMOH, 2015). Children under age five and pregnant women are mostly affected and Africa still bears over 80% of the global malaria burden which Nigeria accounts for about 29% of this burden. Moreover, in combination with the Democratic Republic of Congo, Nigeria contributes up to 40% of the global burden (WHO, 2014).

Malaria infection has been reported to induce acute injuries to vital organs and the most pronounced changes inflicted due to this disease involve the blood, spleen, liver and kidney of the infected host (Vineet and Bagai, 2014). The greatest impact of the disease is on the poor people of the world and most of these populations are found in the rural settings, especially in African communities where the people have poor nutritional status and also lack access to good health facilities (Ajala et al., 2011). Thus, the rural dwellers depend more on herbs and other forms of traditional medicines for cure (Idowu et al., 2010). Various medicinal properties have been attributed to natural herbs (Vaghasiya et al., 2011) and plants constitute the main source of new pharmaceuticals and healthcare products (Ivanova et al.,

2005).

WHO (2016), indicated malaria to be endemic in 91 countries as at the beginning of 2016 and a total funding for malaria control and elimination in 2015 was estimated at US\$2.9 billion. About 212 million cases of malaria occurred worldwide, where 429,000 deaths were recorded globally and 303,000 malaria deaths are estimated to have occurred in children aged under 5, which is equivalent of to 70% of the global total (WHO, 2016).

Plants provide many advantages such as ornament, oxygen, food, beverages, clothing, perfumes and building materials and they are source of an enormous number of compounds, known as 'secondary metabolites' (Laudicina et al., 2013). In Nigeria, various plants are used for the management of malaria and these vary from one locality to another (Aiyeloja and Bello, 2006; Odugbemi et al., 2007). Within the context of traditional practice, malaria is commonly treated with decoctions or infusions from bitter plants (Randrianarivelosia et al., 2003), and the popularity of herbs in traditional medicine has been linked to their higher likelihood of containing pharmacologically active compounds compared to woody plant forms (Thomas et al., 2009).

In the last decade, there has been resurgence in search for new lead compounds from plants to treat malaria (Noronha et al., 2018). A number of traditional herbs have been tested and used in the prevention and treatment of malaria including, leaves of *Carica papaya*, *Azadirachta indica* popularly called Dongoyaro in Nigeria, *Mangifera indica* and *Citrus limon* (Idowu et al., 2010; Gbolahan et al., 2014). Chloroform extract of *C. papaya* have been found to be active against the malarial parasites (Abass et al., 2017).

Etuk et al. (2010) and Ene and Atawodi (2012) reported the use of *M. indica* leaf decoctions in the treatment of malaria. Various parts of *M. indica* tree have been used in traditional medicine for the treatment of different ailments and a number of bioactive phytochemical constituents such as polyphenols, terpenes, sterols, carotenoids, vitamins, and amino acids, and so forth have been reported with several studies proving the pharmacological potential of different parts of mango trees such as leaves, bark, fruit peel and flesh, roots, and flowers as anticancer, anti-inflammatory, anti-diabetic, antioxidant, antibacterial, antifungal, anthelmintic, gastroprotective, hepatoprotective, immunomodulatory, antiplasmodial, and antihyperlipemic (Ediriweera et al., 2017).

A large variety of chemical compounds have been reported in *M. indica* which includes mangiferin, gallic

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acid, catechins, quercetin, kaempferol, protocatechuic acid, ellagic acids, propyl and methyl gallate, rhamnetin, and anthocyanins as the major polyphenolic compounds found in *M. indica* (Nayan et al., 2017). *C. papaya* belongs to the family of Caricaceae. It is commonly called paw-paw and it is known for its food and nutritional values worldwide (Melariri et al., 2012).

The properties of papaya fruit and other parts of the plant are also well known in traditional system of medicine and the medicinal application of papaya makes it a valuable nutraceutical fruit plant. The different parts of the *C. papaya* plant includes leaves, seeds, latex and fruit exhibited to have medicinal value and have been used in the treatment of various ailments. Young leaves are rich in flavonoids (kaempferol and myricetin), alkaloids (carpaine, pseudocarpaine, dehydrocarpaine I and II), phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), and cynogenetic compounds (benzyl glucosinolate). Both the leaf and fruit of *C. papaya* Linn. possess carotenoids namely β -carotene, lycopene, anthraquinones glycoside, as compared to matured leaves; hence, possess medicinal properties like anti-inflammatory hypoglycaemic, anti-fertility, abortifacient, hepatoprotective, wound healing. Recently, its antihypertensive and antitumor activities have also been established (Anjum et al., 2013).

The latex from unripe papaya fruit contains enzymes papain and chymopapain, while vitamin C and E are also constituents of the leaves (Yogiraj et al., 2014). Variety of substances are present in citrus fruits and they include carbohydrates, fibre, vitamin C, potassium, folate, calcium, thiamine, niacin, vitamin B6, vitamin A, phosphorus, magnesium, copper, riboflavin, pantothenic acid and a variety of phytochemicals which are necessary for proper functioning of the body, although some confer additional protection against chronic disease over basic nutrition. In addition, phytochemicals including essential oils, alkaloids, flavonoids, coumarins, psoralens and carotenoids are also present with previous pharmacological studies reporting the antimicrobial, anthelmintic, insect repellent, antioxidant, anticancer, cardiovascular, central nervous, anti-inflammatory, analgesic, antidiabetic, reproductive, gastrointestinal, immunological, respiratory and many other pharmacological effects of the *C. limon* (Al-Snaf, 2016).

Titanji et al. (2008) reported the use of *C. papaya*, *M. indica* as part of the medicinal plants used in the treatment on malaria in Cameroon in the form of decoction, dried powder or ground material prepared from plants for consumption as teas, steam bath or enema which are sold as herbal remedies. Water extracts of pawpaw and mango leaves have been confirmed to show potencies against malaria parasites and they compare favorably with an established long acting orthodox anti-malarial drug, sulphadoxine/pyrimethamine (Gbolahan et al., 2014). Indigenous dwellers also claimed that the usage of selected forest plants leaves and parts

are effective in the management of malaria. This study evaluates the efficacy of a herbal cocktail which contains fruit extract of *C. limon*, aqueous leaf extract of *C. papaya*, *A. indica* and *M. indica* commonly used in the treatment of malaria in some communities in Nigeria, as well as to determine the biochemical or histomorphological changes that could occur in the liver following treatment with the cocktail or extracts contained therein.

MATERIALS AND METHOD

Experimental animals

This experiment was conducted in strict compliance with the humane animal care standards of the University of Benin, Benin City, Nigeria. Six weeks old inbred Wistar rats, weighing an average of 200 g obtained from the Anatomy Department of the University of Benin animal house were used as experimental animals for this study. All animals purchased were kept in ventilated cages in the Animal House at the Anatomy Department, University of Benin, Benin city, Nigeria. They were fed with growers mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo State, Nigeria throughout the duration of this study.

Parasite and infection

Chloroquine-sensitive *Plasmodium berghei* (NK 65 strain) obtained from the Institute of Advance Medical Research and Training (IMRAT), University of Ibadan, Nigeria was used for this study. Thirty-five (35) adult Wistar rats weighing an average of 200 g were divided into seven groups labeled I, II, III, IV, V, VI and VII. Each of the groups consisted of 5 rats, which were allowed to attain the requisite weights before commencement of the experiment.

Group I: Non-infected control group (Negative control) (Distilled water was administered only).

Group II: Infected untreated group (*P. berghei* + Distilled water was administered).

Group III: *P. berghei* + Herbal cocktail was administered.

Group IV: *P. berghei* + *A. indica* extract was administered.

Group V: *P. berghei* + *M. indica* extract was administered.

Group VI: *P. berghei* + *C. papaya* extract was administered.

Group VII: *P. berghei* + *C. limon* extract was administered.

Inoculation procedure

Three donor mice with rising parasitemia of 25% were sacrificed and blood was collected into ethylene diaminetetraacetic (EDTA) bottle and diluted with phosphate buffer saline to 10^8 parasitized erythrocytes/mL. Healthy Wistar rats were inoculated intraperitoneally with preparation of the infected blood (Peter and Anatoli, 1998).

Blood cytology evaluation

A small drop of blood from the tail of each infected rats was collected on clean grease free slide. Thin blood films were stained with Giemsa and were allowed to air-dry and viewed with oil immersion objectives (Akin-Osaniye et al., 2013). The percentage parasitaemia evaluation was determined (total number of pRBC/total number of RBC \times 100) for each infected rat (Innocent et al., 2017). Rats with parasitaemia, at least equal to 25%, were

either treated with the cocktail or each constituent extract on the seventh post-infection day.

Plant

Cocktail and individual extract

The plant samples collected were identified by a plant Taxonomist from Plant Biology and Biotechnology Department of the University of Benin, Benin City, Nigeria as *A. indica* (family Meliaceae), *M. indica* (family Anacardiaceae), *C. papaya* (family Caricaceae), and *C. limon* (family Rutaceae) (Arzoo and Parina Kumari, 2017). The cocktail, *C. limon* juice extract and aqueous leaf extracts of *M. indica* and *C. papaya* were administered to respective groups at a dose of 100 mg/kg/body weight, while aqueous leaf extract of *A. indica* was administered at 10 mg/kg/body. The chemotherapeutic effect of the extract against *P. berghei* was investigated after seven days of administration. Phytochemical studies were carried out at the Pharmacognosy Department of the University of Benin, Benin City, Nigeria to determine the presence of carbohydrate, alkaloid, flavonoid, tannins and saponin in aqueous leaf extract of *A. indica*, *M. indica*, *C. papaya* and fruit extract of *C. limon*, using standard procedures proposed by Sofowora (1982) and Evans (2002).

Histopathology studies

The liver was excised and immediately transferred into 10% neutral buffered formalin and processed for light microscopic study, using an automatic tissue processor machine (Shandon 2000, Leica, Frankfurt, Germany). Tissues were dehydrated in various grades of alcohol then cleared in two changes of xylene, infiltrated in two changes of wax bath and finally embedded in paraffin wax. Five microns thick paraffin sections were obtained, which were finally stained using the Hematoxylin and Eosin staining procedure and the sections mounted with DPX and examined microscopically by means of x10 and x40 objective lenses (Avwioro, 2014).

Biochemical studies

Biochemical markers associated with liver functions were determined using the plasma obtained from the Wistar rats upon necropsy after seven days of treatment. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein and albumin were analyzed using commercial diagnostic kits from Randox laboratory, United Kingdom. The kits employed the procedure of Reitman and Frankel (1957) for the analysis of AST and ALT, while total protein was estimated using the procedure of Tiez (1995). Albumin was estimated using the method of Grant (1987).

Statistical analysis

Data analysis was performed using statistical package for social sciences (SPSS) version 20.0. Data were expressed as mean \pm standard deviation (SD). Test of significance was calculated using paired Student's t-test. $p < 0.05$ was considered to be significant.

RESULTS

Histopathological findings

Histopathological findings indicated that extracts of *C.*

papaya and *C. limon* were the most efficacious as there was significant reduction in the malaria associated liver pathology; while the groups administered with aqueous leaf extract of *M. indica* was observed to be least potent. Sections of uninfected rats administered with distilled water only showed normal histological features composed only of hepatocytes, portal vein and sinusoids, the morphology of the hepatocytes appeared normal and the sinusoids were not infiltrated; and no pathological lesion observed (Figure 1). Liver sections of the infected untreated group showed very poor architecture. There is severe portal triaditis and the portal tracts showed periportal infiltration of inflammatory cells with mild congestion of the portal vein, some of the hepatocytes showed foamy cytoplasm and some showed cellular debris engulfed by Kupffer cells. Heavy infiltrates of inflammatory cells and mild vascular congestion was observed. Parasites were engulfed in Kupffer cells and malaria pigment (haemozoin) was also present (Figure 2). Liver sections of infected rats administered with the cocktail showed sinusoids mildly packed with parasite-laden Kupffer cells and malaria pigment, mild inflammatory cellular infiltrates were also observed to be present around the portal zone, the hepatic portal vein was not congested (Figure 3). Liver sections of infected rats treated with aqueous leaf extract of *A. indica* showed decreased inflammatory cellular infiltrates indicating resolving malaria infection; however, the sinusoids were mildly packed with Kupffer cells which have engulfed haemozoin pigment and cellular debris and mild congestion was observed within the portal vein (Figure 4). Comparison with the untreated infected group administered with distilled water only showed no observable congestion in the blood vessels and there was significant reduction in the polymorphonuclear cellular infiltrates (Figure 4). Liver sections of infected rats treated with the aqueous extract of *M. indica* showed sinusoids moderately packed with parasite-laden Kupffer cells and cellular debris (Figure 5). There was a mild observable congestion in the portal vein with severe periportal infiltration by inflammatory cells, and various regions of the liver were packed with haemozoin. The morphology of the hepatocytes showed micro vesicular steatosis and the cytoplasm are vacuolated and infiltrated with fat. Few cells with engulfed parasites are also seen (Figure 5). Infected group administered with aqueous leaf extract of *C. papaya* showed normal central venules without congestion. There is no observable congestion of the hepatic portal vein and the hepatocytes appeared normal. Mild sinusoidal Kupffer cell activation is present; however, the central venules is not congested and the sinusoids appeared normal and not infiltrated, no pathological lesion seen (Figure 6) Liver sections of infected group treated with fruit extract of *C. limon* showed normal morphology of most of the hepatocytes, however, with a few engulfed parasites. There was no portal vein congestion, liver sinusoids were mildly packed

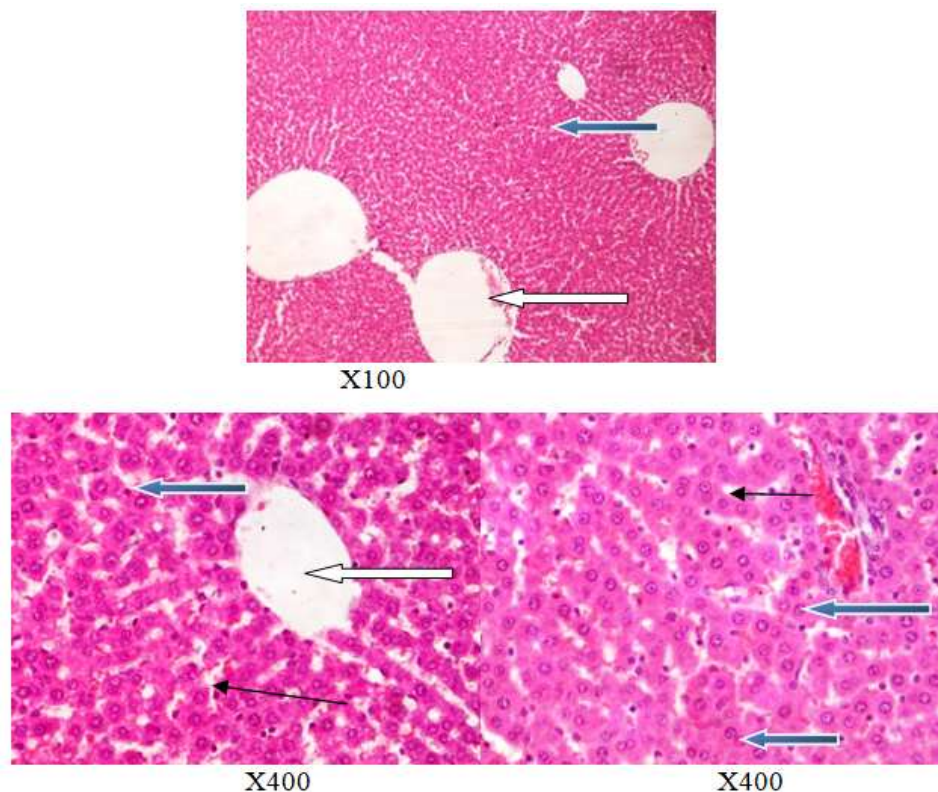


Figure 1. Photomicrograph of a liver section of uninfected rats administered with distilled water only showing normal central venules without congestion (white arrow), the morphology of the hepatocytes appear normal (blue arrow), the sinusoids appear normal and not infiltrated (slender arrow), no pathological lesion seen.

with Kupffer cells and haemozoin pigment was seen in various regions (Figure 7).

Biochemical studies

Table 1 shows the mean and standard deviation of liver enzymes, albumin and total protein obtained in all groups. Table 2 shows the comparative analysis of liver enzymes, albumin and total protein obtained in all groups administered with the cocktail or either extract contained therein. There were statistical significant variations in ALT activity ($P < 0.0001$) when all groups administered with the cocktail or either of the constituent extract were compared with the non-infected control group administered with distilled water only. AST activities and albumin values were observed to be normal among all groups ($P > 0.05$), while rats administered with the cocktail showed increase in total protein values ($P < 0.04$). Table 3 shows the comparative analysis of liver enzymes, albumin and total protein between the infected untreated groups and groups administered with the cocktail or either extracts showed no increase in albumin values and AST activity was observed to be

normal ($P > 0.05$). However, there was a significant increase in ALT activities in rats administered with either of the cocktail ($P < 0.0003$), *A. indica* ($P < 0.0008$) or *C. papaya* ($P < 0.01$). Significant increase was observed in the total protein values among rats administered with the cocktail ($P < 0.04$) when compared with the infected untreated group administered with distilled water only. Comparative analysis of liver enzymes, albumin and total protein between the cocktail group and groups administered with various individual plant extract showed no significant increase in AST activity ($P > 0.05$). However, significant increase in ALT activity ($P < 0.0001$) was observed in other groups. Significant increase was observed in the groups administered with *A. indica* ($P < 0.03$) and in the total protein values of groups administered with aqueous leaf extract of *C. papaya* ($P < 0.001$) shown in Table 4. In addition, comparative analysis of liver enzymes, albumin and total protein between groups administered with individual extracts showed no statistical significant increase in the albumin values and AST activities; however, there was variations in the ALT activities ($P < 0.00001$) with the exception of rats administered with aqueous leaf extract of *M. indica* or *C. limon* ($P > 0.05$) while increase in total protein

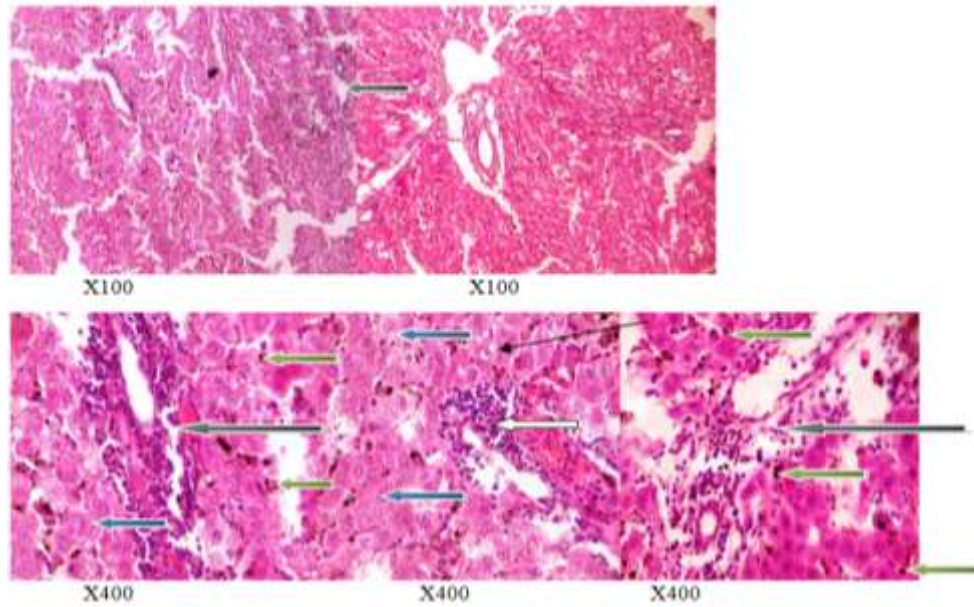


Figure 2. Photomicrograph of a liver section of Rat induced with *Plasmodium berghei* showing very poor architecture. Sinusoids are densely packed with malaria pigments (haemozoin) and cellular debris engulfed by Kupffer cells, there is severe portal triaditis; the portal tracts show periportal infiltration of inflammatory cells with mild congestion of the portal vein (white arrow), some of the hepatocytes show foamy cytoplasm (blue arrow) and some show engulfed parasites (green arrow). The sinusoids appear normal and not infiltrated (slender arrow).

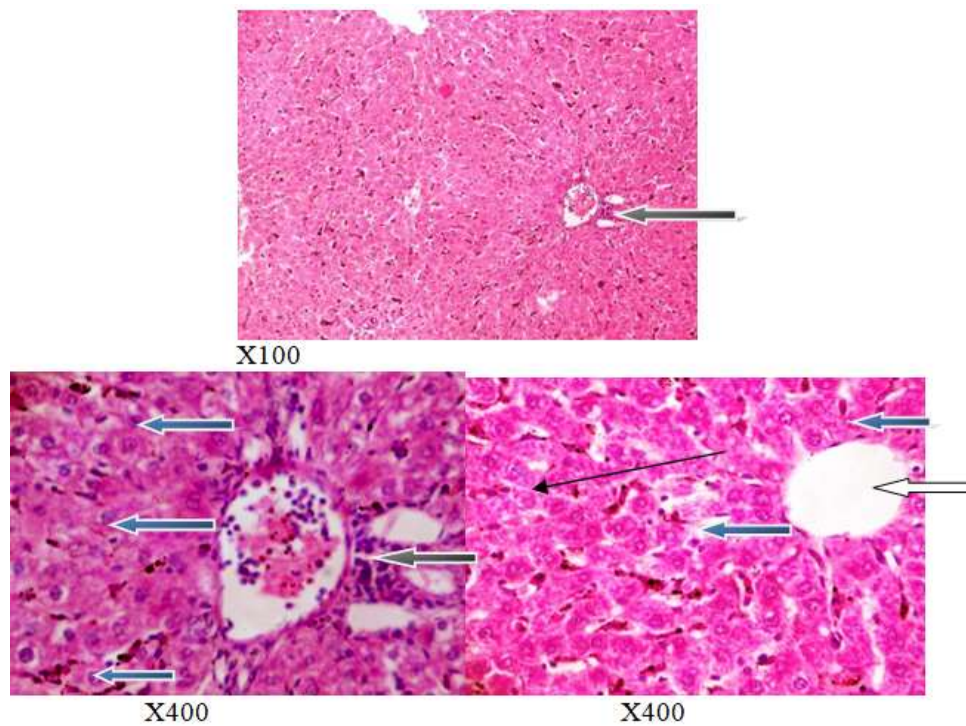


Figure 3. Photomicrograph of a rat liver infected with *P. berghei* and treated with the herbal mixture (Cocktail) showing sinusoids mildly packed with parasite laden Kupffer cells, normal central venules without congestion (white arrow), however, the portal tract show mild infiltration of inflammatory cells-mild portal triaditis (black arrow), the morphology of the hepatocytes appear normal (blue arrow), the sinusoids appear normal and not infiltrated (slender arrow).

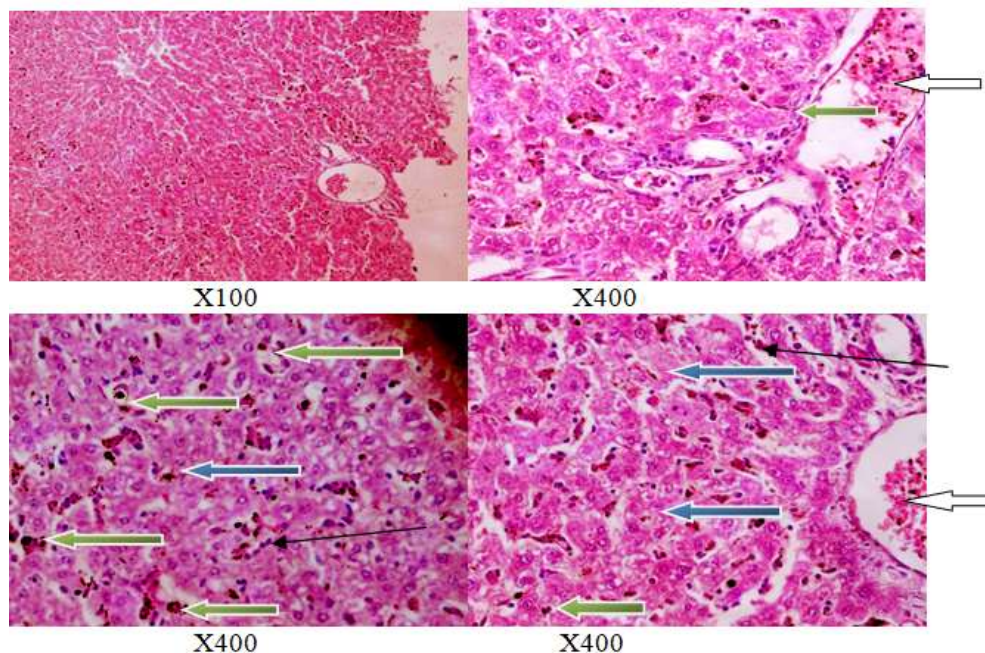


Figure 4. Liver section of Rat induced with *P. berghei* and treated with 10 mg/kg of aqueous leaf extract of *A. indica* showing sinusoids mildly packed with Kupffer cells which have engulfed haemozoin pigments and cellular debris. There is mild congestion within the portal vein (white arrow), the morphology of the hepatocytes show vacuolated and spongy-like cytoplasm (blue arrow) as well as engulfed parasites, the sinusoids are mildly infiltrated (slender arrow).

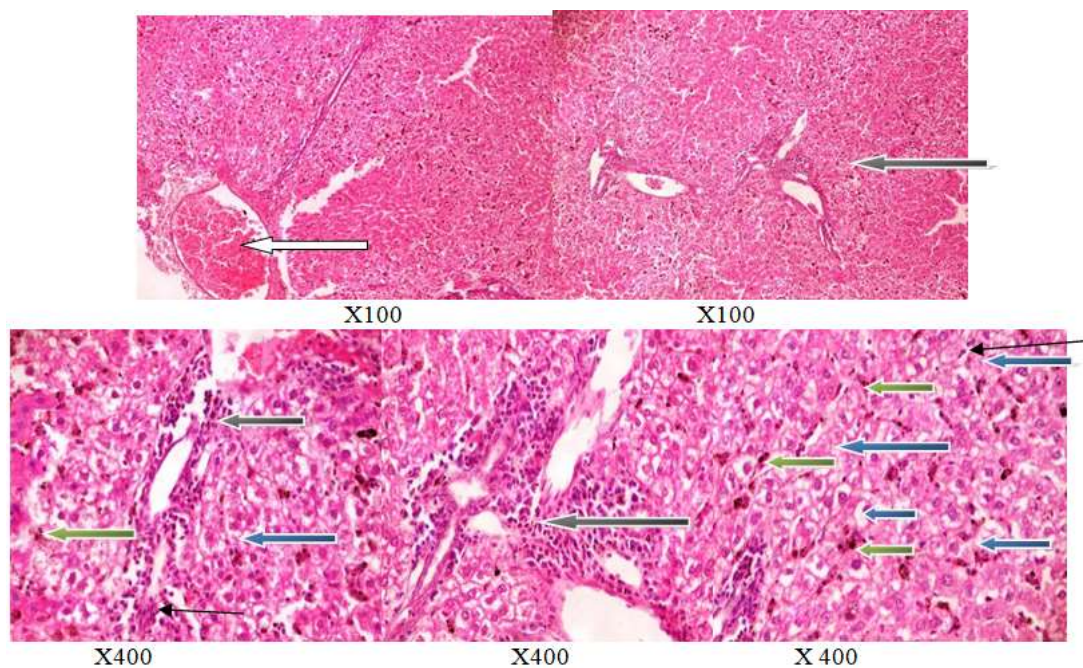


Figure 5. Rat liver infected with *P. berghei* and treated with *M. indica* showing sinusoids moderately packed with Kupffer cells laden parasites and cellular debris, mild congestion within the portal vein (white arrow) severe periportal infiltration by inflammatory cells (black arrow), the morphology of the hepatocytes show microvesicular steatosis; the cytoplasm is vacuolated and infiltrated by fat (blue arrow). Few cells with engulfed parasites are seen (green arrow).

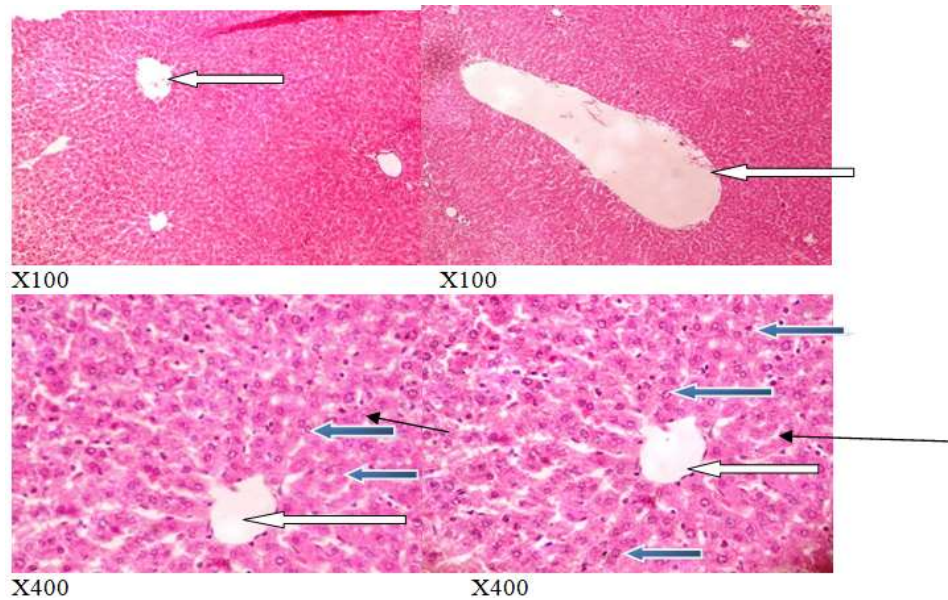


Figure 6. Rat liver infected with *P. berghei* and treated with *C. papaya* showing mild sinusoidal Kupffer cell activation. The central venules is not congested (white arrow), the morphology of the hepatocytes appear normal (blue arrow), the sinusoids appear normal and not infiltrated (slender arrow), no pathological lesion seen.

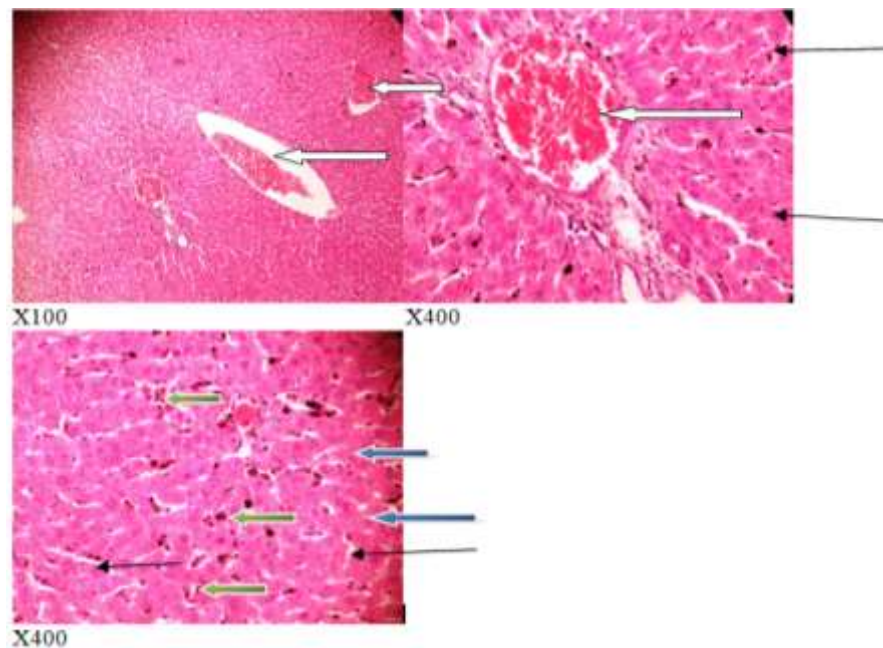


Figure 7. Rat liver infected with *P. berghei* and treated *C. limon* showing, mildly packed Kupffer cells, the portal vein is mildly congested (white arrow), the morphology of most of the hepatocytes appear normal (blue arrow) while other few show engulfed parasites (green arrow), the sinusoids appear normal (slender arrow).

values was observed in rats administered with either *M. indica* or *C. papaya* <0.002 and groups administered with *C. papaya* or *C. limon* ($P < 0.02$) (Table 5).

Phytochemical studies

The results obtained from the phytochemical analysis of

Table 1. Mean and standard deviation of liver enzymes, albumin and total protein obtained in all groups.

Parameter	Non-Infected control	Infected untreated group	Cocktail	<i>A. indica</i>	<i>M. indica</i>	<i>C. Papaya</i>	<i>C. limon</i>
AST (U/L)	83±6.8	92±9.0	91±8.0	83±21.0	93±15.0	90±16	96±23.0
ALT (U/L)	28±0.8	29±1.4	47±1.9	51±0.5	33±2.7	39±2.6	33±1.5
ALB (g/dl)	4.1±0.1	4.0±0.2	4.0±0.1	4.2±0.1	4.1±0.1	4.1±0.2	4.1±0.1
TP (g/dl)	6.3±0.3	6.3±0.2	6.5±0.1	6.4±0.3	6.5±0.1	6.3±0.1	6.4±0.1

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALB: albumin; TP: total protein.

Table 2. Comparative analysis of the liver enzymes, albumin and total protein obtained in all groups administered with the cocktail or various individual extracts.

Parameter		Non-Infected control vs. Infected untreated group	Non-Infected control vs. Cocktail	Non-Infected control vs. <i>A. indica</i>	Non-Infected control vs. <i>M. indica</i>	Non-Infected control vs. <i>C. papaya</i>	Non-Infected control vs. <i>Citrus limon</i>
AST (U/L)	"t"	0.10	-1.77	0.04	-1.37	-0.87	-1.18
	"p"	0.17	0.06	0.48	0.11	0.21	0.14
ALT (U/L)	"t"	-0.43	20.68	-54.20	-3.79	-9.04	-7.09
	"p"	0.34	0.0001***	0.0001***	0.003**	0.0001***	0.0005***
ALB (g/dl)	"t"	1.12	1.58	-1.0	0.41	0.00	1.0
	"p"	0.15	0.08	0.17	0.35	0.5	0.17
TP (g/dl)	"t"	-0.14	-1.92	-0.69	1.50	0.36	-0.77
	"p"	0.45	0.04*	0.26	0.09	0.36	0.18

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALB: albumin; TP: total protein. *Values are considered statistically significant. (*p<0.05) relative to the non-infected control group. t= (student's t-test).

M. indica, *C. papaya*, *A. indica* and *C. limon* extract showed the presence of some secondary metabolite like carbohydrate, alkaloid, flavonoid, tannins and saponin. The presence of these secondary metabolites in this extract may be responsible for the antiplasmodial activity of the various extract (Table 6).

DISCUSSION

This study indicated that the herbal cocktail and individual extract contained therein has antiplasmodial effect on *P. berghei*. Histological studies of the infected rats showed recovery of the hepatocytes from congested black pigmentation (haemozoin pigment) as induced by these plant extracts; thus highlighting their importance in traditional treatment of malaria. Examination of the liver section clearly indicate that the administration of aqueous leaf extract of *C. papaya* and fruit extract of *C. limon* significantly reduced malaria associated with liver pathology when compared with the infected untreated group administered with distilled water only. Rats

administered with the cocktail and plants extracts of *A. indica* also showed ameliorative effect on malaria associated liver pathology; while the group administered with aqueous leaf extract of *M. indica* was observed to be least potent. Photomicrographs of the liver of the uninfected control group administered with distilled water appeared normal as no endemic inflammation of hepatocytes was observed. Liver changes in severe malaria reported by Whitten et al. (2011) were observed in the untreated infected group administered with distilled water only. Sinusoids was observed to be densely packed with malaria pigments (haemozoin) and cellular debris engulfed by Kupffer cells. Heavy infiltrates of inflammatory cells was also confirmed to be present as observed by Olayode et al. (2015), mild vascular congestion of the hepatic portal vein was observed. The sinusoids of the infected rats treated with the cocktail was observed to be mildly packed with parasite laden Kupffer cells and haemozoin, a mild inflammation was also observed around the portal zone. The hepatic portal vein was not congested. Infected rats treated with aqueous leaf extract of *A. indica* showed decreased inflammatory

Table 3. Comparative analysis of liver enzymes, albumin and total protein between the infected untreated group and groups administered with the cocktail and individual extracts.

Parameter		Infected untreated vs. Cocktail	Infected untreated vs. <i>A. indica</i>	Infected untreated vs. <i>Mangifera indica</i>	Infected untreated vs. <i>C. papaya</i>	Infected untreated vs. <i>Citrus limon</i>
AST (U/L)	"t"	-1.18	0.74	-0.07	0.22	-0.27
	"p"	0.14	0.24	0.47	0.42	0.40
ALT (U/L)	"t"	-5.36	-6.64	-0.98	-2.79	-1.21
	"p"	0.0003***	0.0008***	0.18	0.01*	0.13
ALB (g/dl)	"t"	0.00	-1.63	-0.87	-0.87	-0.69
	"p"	0.5	0.07	0.20	0.20	0.26
TP (g/dl)	"t"	-1.93	-0.60	-1.47	0.6	0.88
	"p"	0.04*	0.28	0.09	0.28	0.20

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALB: albumin; TP: total protein. *Values are considered statistically significant. (*p<0.05) relative to the infected untreated group. t= (student's t-test).

Table 4. Comparative analysis of liver enzymes, albumin and total protein between the cocktail group and groups administered with various individual plant extract.

Parameter		Cocktail vs. <i>A. indica</i>	Cocktail vs. <i>M. indica</i>	Cocktail vs. <i>C. papaya</i>	Cocktail vs. <i>C. limon</i>
AST (U/L)	"t"	0.86	-0.22	0.21	-0.41
	"p"	0.21	0.42	0.42	0.35
ALT (U/L)	"t"	-4.08	9.84	5.82	12.89
	"p"	0.002**	0.0001***	0.0002***	0.0001*
ALB (g/dl)	"t"	-2.14	-1.21	-1.05	-1.0
	"p"	0.03*	0.13	0.16	0.17
TP (g/dl)	"t"	0.81	0.77	4.32	1.43
	"p"	0.22	0.23	0.001*	0.09

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALB: albumin; TP: total protein. *Values are considered statistically significant. (*p<0.05) relative to the infected untreated group. t= (student's t-test).

cellular infiltrates in the liver sections; indicating that infection is resolving, which is in line with the observation of Anyaechie (2009) who documented that an active ingredient Irocin A isolated from Neem leaves is toxic to causative strains of malaria. Comparison with the infected untreated group administered with distilled water only showed no observable congestion in the blood vessels, and there was significant reduction in the polymorphonuclear cellular infiltrates.

The sinusoids of infected rats administered with aqueous extract of *M. indica* were moderately packed with parasite-laden Kupffer cells and cellular debris; mild observable congestion in the portal vein and various

regions of the liver were packed with haemozoin. The infected group treated with aqueous leaf extract of *C. papaya* had mild sinusoidal Kupffer cell activation and haemozoin pigments were observed in various regions of the liver section. Relatively few cellular infiltrates was observed to be present, indicating a resolving plasmodial infection. There was no observable congestion of the hepatic portal vein. This observation is in line with Longdet and Adoga (2017) who observed that extract of *C. papaya* leaf confers a dose dependent decrease on the level of parasitaemia when compared to the non-infected control group. This study corroborates Arise et al. (2012) who reported that extract of *C. papaya* gave

Table 5. Comparative analysis of liver enzymes, albumin and total protein between groups administered with individual extracts.

Parameter		<i>A. indica</i> vs. <i>M. indica</i>	<i>A. indica</i> vs. <i>C. papaya</i>	<i>A. indica</i> vs. <i>C. limon</i>	<i>M. indica</i> vs. <i>C. papaya</i>	<i>M. indica</i> vs. <i>C. limon</i>	<i>C. Papaya</i> vs. <i>C. limon</i>
AST (U/L)	"t"	-0.89	-0.60	-0.94	0.34	-0.23	-0.49
	"p"	0.20	0.28	0.19	0.37	0.41	0.32
ALT (U/L)	"t"	14.86	10.22	25.40	-3.71	-0.43	4.20
	"p"	0.0001**	0.00001*	0.0001*	0.003*	0.34	0.002*
ALB (g/dl)	"t"	1.26	0.45	1.77	-0.23	0.45	0.49
	"p"	0.12	0.27	0.68	0.41	0.33	0.32
TP	"t"	-0.42	1.16	0.00	4.02	0.79	-2.53
	"p"	0.34	0.14	0.5	0.002*	0.23	0.02*

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALB: albumin; TP: total protein. *Values are considered statistically significant. (* $p < 0.05$) relative to the infected untreated group. t= (student's t-test).

Table 6. The phytochemical constituents of the aqueous leaf extract of *M. indica*, *C. papaya*, *A. indica* and *C. limon*.

Phytocompound	<i>M. indica</i>	<i>C. papaya</i>	<i>A. indica</i>	<i>C. limon</i>
Carbohydrate	++	+	++	+
Alkaloid	+	++	++	+
Flavonoid	+	+	++	+
Tannins	++	+	+	++
Saponin	+	+	+	+

+: Present in low concentration; ++: present in moderate concentration.

significant suppression ($P < 0.05$) of parasitemia, following five days administration in established infection. Fatmawaty et al. (2017) further buttressed the antiplasmodial activity of *C. papaya* in *P. berghei* infected mice and histological examination of liver tissues of treated and untreated mice; which further supports the potential antimalaria of this plant, hence validating its traditional use in the treatment of malaria. Thomas et al. (2004), Fatmawaty (2013) and Okpe et al. (2016) also reported the antilarvicidal and antimalarial potential of fruit and leaf extract of *C. papaya*, both *in vitro* and *in vivo*. Liver sections of the group treated with *C. limon* showed reduced inflammatory cellular infiltrates, and the sinusoids were mildly packed with Kupffer cells and haemozoin pigment was seen in various regions. There was no portal vein congestion observed.

Observation in this study further corroborates Saganuwan et al. (2014) who indicated that many Nigerian plants can be used for the treatment of malaria as the groups administered with the cocktail or either extracts of *A. indica*, *M. indica*, *C. papaya* or *C. limon* and they showed reduction in the liver pathology associated with malaria infection. The leaves used in this study (*C. papaya*, *A. indica*, *M. indica*) showed the presence of

phytochemical constituents, including flavonoid, alkaloid and tannin which compounds are potentially used for various treatments such as herbal remedies (Longdet and Adoga, 2017). This study corroborates the observation of some studies (Dhar et al., 1996; Mulla and Su, 1999; Nathan et al., 2005) that showed that azadirachtin and other limonoids available in neem extracts are active on malaria vectors. Furthermore, observation in these study are in line with Osanaiya et al. (2013) who confirmed that antimalarial activity of *A. indica* leaf extract in *P. berghei* infected albino mice.

Biochemical studies indicated that the cocktail and individual extracts do not alter the AST activity in the infected rats administered with the cocktail or that of the constituent extracts contained therein when compared with the non-infected group administered with distilled water only. However, there was an observable increase in the activity of ALT in the rats treated with the cocktail or that of the extract. Rats administered with aqueous leaf extract of *M. indica* showed a reduction in ALT activity compared to those administered with the cocktail or either of the extracts of *A. indica*, *C. papaya* or *C. limon*. Thus, the result obtained in this study agrees with the findings of Olayode et al. (2017) who observed that

M. indica has an ameliorative effect against hepatocellular injury. There was variation in the AST among the rats in the different group but no significant increase was observed in the enzyme activity ($p > 0.05$). Also, there was no significant change in albumin levels $P > 0.05$ and total protein values among groups administered with *A. indica*, *M. indica*, *C. papaya* and *C. limon* were observed to be normal, while rats administered with the cocktail showed a significantly raised albumin level. AST activity in the infected groups administered with cocktail or that of the extracts remained normal when compared with the infected group administered with distilled water only; there was also no significant difference in the ALT activity among rats in the groups treated with *M. indica* or *C. limon* when compared with the infected untreated group administered with distilled water.

Additionally, no difference in the total protein and albumin values were observed in test groups compared to the untreated infected group administered with distilled water; however, the group administered with the cocktail showed an elevated total protein concentration. Comparison of *A. indica*, *M. indica*, *C. papaya* and *C. limon* extracts with cocktail group showed no significant difference in the AST activity. Notwithstanding, an increase in ALT activity in various groups administered with either of the extract when compared to the group administered with the cocktail was observed. No observable difference in the albumin level between the infected rats administered with the cocktail and groups administered with either of the plant extracts, with the exception of the groups administered with the aqueous leaf extract of *A. indica*, showed an increased level of albumin when compared to the group administered with cocktail.

Comparative analysis of total protein values among infected rats in the group administered with cocktail and infected rats administered with either extracts of *A. indica*, *M. indica*, *C. papaya* or *C. limon* showed no significant difference, with the exception of the rats in the group treated with aqueous leaf extract of *C. papaya* that showed an increase in total protein value when compared with the group administered with the cocktail. No observable difference in the AST activity between various individual extracts when statistically compared with one another. Significant elevation of ALT activity induced by individual extracts was observed.

In addition, increased activity of ALT enzyme in the groups administered with *M. indica*, *C. papaya* and *C. limon* when compared with the groups treated with aqueous leaf extract of *A. indica* was also observed. Furthermore, there was increase in ALT activity in groups treated with *C. papaya* upon comparison with the groups administered with *M. indica*; while the activity in the group treated with *C. limon* was observed to be insignificant ($P > 0.05$). Increase in ALT activity occurred in groups administered with *C. limon* fruit extract when compared with the group administered with aqueous leaf extract of

C. papaya; while albumin levels among the various groups treated with various extracts showed no significance. There was increase in the total protein concentration in the group treated with *C. papaya* compared to the group treated with *M. indica*. Increase in total protein values in the groups treated with *C. limon* when compared with the groups administered with aqueous leaf extract of *C. papaya* was as well observed.

Conclusion

This study indicated that the cocktail and individual plant extracts contained therein possess antiplasmodial effect by inducing recovery of hepatic cells from congested black pigmentation (haemozoin pigment) and reduction in malaria associated liver pathology. Thus, observation in this study gave credence to the traditional use of the plants for the treatment of malaria.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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