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Hepato-Protective Assessment of Pawpaw Leaves, Neem, Lemon Grass and Acts on Plasmodium Berghei Parasitized Wistar Rats

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ABSTRACT

Malaria is a major concern in Nigeria, and stands as the second leading cause of death from all infectious disease in Africa. Several studies have reported the damaging effect of the parasite to various body organs especially the liver. Reports over time has shown the benefits of various plants extracts in ethno-medicine. However, not much have been done on the effects of some of these extracts in combined form on its hepato-protective assessment in comparison with any known ACT based anti-malaria. The focus of this study was to explore the hepato-protective properties of ethanoic extract of *Carica papaya Linn*, *AzadirachtaIndica, CymbopogonCitratus*against ACT based antimalarial therapy on plasmodium berghei parasitized wistar rats. Phytochemical analysis of the extracts were done according to the method described by *Treaseand Evans*. Hepato-protective assessment were done using the liver function tests and assay of the liver histology respectively. One hundred and ten (110) rats distributed into 11 groups, each group having 10rats were used for the experiment. Negative control received just feed and water, Positive control were induced with the malaria parasite and given feed and water only. The tests groups were induced with malaria, received feed and water and treated with 500mg/kg, 250mg/kg and 165mg/kg doses of the extracts, both individually and in combined forms, as well as the standard ACT anti-malaria. Phytochemical screening showed that the plant extracts possessed high concentration of Tannins, Flavonoids, Saponins and Alkaloids. Plasmodium berghei increased the activities of ALP, ASP and ALT when compared with the positive control group. This may be attributed to increase in functional capacity of the liver as a result of the presence of the infection for the tests groups. Treatment with the plant extracts decreased ALP and ALT levels significantly ($P<0.05$), as well as AST levels except for the Neem extract. Histological examination of the liver of test animals showed no extensive damage to the tissue by the individual extracts when compared to the negative control group.

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KEYWORDS: *Hepato-protective, AzadirachtaIndica, Carica papaya Linn, CymbopogonCitratus, ethanol extract, plasmodium berghei, ACTs*

INTRODUCTION

Malaria is a mosquito-borne disease predominate in the tropics and caused by a plasmodium parasite. It is a major concern in Nigeria, and stands as the second leading cause of death from all infectious disease in Africa. Malaria is one of the major killer diseases in Africa causing more than 1 million deaths every year. In Nigeria, the infection rate has been described as holo-endemic (*Salako et al., 1994*) with more than 75% of children aged 2-9 years infected. Malaria is becoming more difficult to manage particularly in areas of multi-drug resistance.

Various orthodox pharmacological options has been used over time in tackling this disease. They include drugs like Chloroquine, Quinine, and Artemisinin derivatives like Artesunate, Artemether, Arteether as well as others. One major problem that arise from the use of these drugs has been issues of resistance, and the fact that most anti-malaria drugs are not affordable by People. This necessitates the shift to self-medication using medicinal plants (*Arese, 2001; Muregi et al., 2003*). Herbs had been used by all cultures throughout history. Plants have always been

a component of mankind's healthcare system. This is either directly or indirectly. Directly, the plant parts like leaves, fruits, stem, bark, roots etc. or even the whole plant are themselves used in the treatment of illnesses. They are the first line treatment for many of the world's population, being readily available, traditional and relatively inexpensive (*Olaniyi, 1998; Okpara et al., 2007*).

A number of traditional herbs have been tested and used in the prevention and also treatment of malaria including Artemisia annua (*Akininyi et al, 1986*), old leaves of *Carica papaya*, roots and leaves of Guinensis, unripe fruit of Capsicum frutescence and *Azadirachtaindica* popularly called Dangoyaro in Nigeria. There is high reliance on traditional medicine in the treatment of malaria, most of which are prepared from plants and available at affordable prices (*Alves and Rosa, 2007*). Ethnobotanical studies (*Odugbemi et al., 2007; Ajibesin et al., 2008; Titanji et al., 2008)* have been carried out on medicinal plants useful in treating malaria in Africa, Nigeria inclusive.

Mature leaves of *Carica papaya* (Caricaceae; Common name: pawpaw) is widely used to treat malaria and splenomegaly (*Adjanohoun JE, Aboubakar N, Dramane K, Ebot ME, Ekpere JA, Enoworock EG, et al 1996*). The essential oils of *Cymbopogoncitratus* were found to produced 86.6% suppression in growth of Plasmodium berghei when compare to a standard drug chloroquine (*Tchoumbougnang, F; Zollo 2005)* Numerous biological and pharmacological activities have been reported of *AzadirachtaIndica* including antibacterial, antifungal and anti-inflammatory activities*(A. Kher et al,1997*).

There is inadequate information in literature on the hepato-protective activities associated with the ethanol extract of Carica papaya Linn, CymbopogonCitratus and AzadirachtaIndica. This study was designed to explore the hepato-protective abilities of these various plant extracts against ACTs treated plasmodium berghei parasitized wistar rats.

MATERIALS AND METHODS

Plant Materials and Preparation of Extracts: Fresh leaves of pawpaw, neem and lemon grass were collected, identified (in the pharmacology department of NnamdiAzikiwe University, Agulu), washed and dried in the oven at 60^{o}C for a period of 45 hours. Leaves were further dried at ambient temperature for a period of two weeks, and then grounded. 250g each of the powdered leaves were dissolved separately in 1000ml of 98% ethanol and allowed for 48hours at room temperature after which it was sieved using porcelain clothe. It was filtered further using a filter paper No 1. The filtrate was concentrated using digital rotary evaporator (TT-52 technel and technel USA) and was dried using thermostat oven (DHG-9023A PEC medicals USA) into a gel like substance and stored individually in a refrigerator (NEXUS).

LETHALITHY (LD50)TEST: The mean lethal dose LD50 of the extracts was determined using *Lorkes* method as described in *(1983)* and modified by *Imafidon et al, (2015)*. A total of 17 wistar rats were used (9 in phase one and 8 in phase two). The extracts were administered via oral routes and were closely monitored for 24 hours for physical signs of toxicity such as gasping, palpitation, sluggishness etc.

PHASE 1: 3 groups with 3 rats each

Group one received 10mg/kg of the extract.

Group two received 100mg/kg of the extract.

Group three received 1000mg/kg of the extract.

PHASE 2: 4 groups with 2 rats each.

Group one received 750mg/kg of the extract

Group two received 1500mg/kg of the extract

Group three received 3000mg/kg of the extract

Group four received 6000mg/kg of the extract.

PREPARATION OF EXTRACT AND DOSE OF STANDARD DRUG:

$$\frac{\text{Average weight of animals (Kg) x Dose (mg/ml)}}{\text{Stock solution (mg/ml)}}$$

EXPERIMENTAL ANIMALS AND TREATMENTS: A hundred and ten (110) male rats between 100g-250g in weight were used for this study. They were obtained from the animal house at the college of medicine, university of Nigeria and kept in well aerated laboratory cages in the Human physiology department animal house Nnewi, with dark and light cycles of 12hrs each observed, fed with water and rat feed (from vital feeds company).The rats acclimatized for a period of two weeks prior to the commencement of the study, and were handled in adherence to the guidelines and recommendation of the ethics committee on the use of animals for research of NnamdiAzikiwe University Awka, Anambra, Nigeria. They were distributed into 11 groups of 10 rats each.

Group 1 (Positive control): Feed and water only.

Group 2 (Negative control): Feed, water and 0.2ml malaria parasite via intraperitoneal route.

Group 3 (Lemon grass extract): Feed, water, 0.2ml malaria parasite and 500mg/kg lemon grass extract.

Group 4 (Pawpaw leaf extract): Feed, water, 0.2ml malaria parasite and 500mg/kg pawpaw leaf extract.

Group 5 (Neem extract): Feed, water, 0.2ml malaria parasite and 500mg/kg neem extract.

Group 6 (Neem and pawpaw leaf extract): Feed, water, 0.2ml malaria parasite and 250mg/kg of neem and pawpaw leaf extract each.

Group 7 (Neem and Lemon grass extract): Feed, water, 0.2ml malaria parasite and 250mg/kg of neem and lemon grass extract each.

Group 8 (Neem, Pawpaw leaf and Lemon grass extract): Feed, water, 0.2ml malaria parasite and 165mg/kg of neem, pawpaw leaf and lemon grass extract each.

Group 9 (Pawpaw leaf and lemon grass extract): Feed, water, 0.2ml malaria parasite and 250mg/kg of pawpaw leaf and lemon grass extract each.

Group 10 (Artemether/Lumefantrine): Feed, water. 0.2ml malaria parasite and 4mg/kg Artemether/lumefantrine.

Group 11 (Dihydoartemisinine/piperaquine phosphate): Feed, water, 0.2ml malaria parasite and 4mg/kg dihydroartemisinine/piperaquine phosphate.

All treatments were done for 7days with the extracts and ACT administered orally.

PHYTOCHEMICAL SCREENING: The ethanoic extracts of the plants were subjected to preliminary phytochemical analysis by methods described by *Trease and Evans (1983)* to test for alkaloids, flavonoids, and glycosides, reducing sugars, steroids, saponins and tannins.

LIVER ENZYMES ASSAY: The activities of Alkaline phosphatase (ALP), Aspartate amino transferase(AST) and Alanine aminotransferase (ALP) were evaluated using the method as described by WHO (2004).

LIVER SACRIFICE AND HISTOLOGY: Liver tissue were harvested from test animals seven days after treatment. Tissue sections were brought to distilled water, stained with Erhlich'shaematoxylin for 30 minutes and rinsed in running water. After drying out, the tissue were differentiated with 1% acid alcohol until only nuclei were stained. Thereafter, it was rinsed in running water and 'blued' in scott's tap water substitute for 3minutes. The sections were further rinsed in tap water, stained with Eosin for 2 minutes, dehydrated, cleared and viewed under a microscope.

DATA ANALYSIS: Results obtained were expressed as mean ± SEM as described by *(Duncan et al., 1997)*. The data were statistically analyzed using one-way analysis of variance (ANOVA) with Turkey's multiple comparison post hoc test to compare the level of significance between the test groups. The values of $p<0.05$ were considered as significant for differences in means.

RESULTS:

Table 1: Phytochemical analysis of the ethanoic extract of pawpaw leaf, lemon grass and neem.

PHYTOCHEMICALS	LEMON GRASS	PAWPAW LEAF	NEEM
Tannins	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Glycosides	-	+	-
Alkaloids	+	+	+
Steroids	+	-	+

Key: + shows presence of phytochemicals,
- Shows absence of phytochemicals.

Table 2: ACUTE LETHAL DOSE OF PLANT EXTRACTS.

DOSE	NEEM	LEMON GRASS	PAWPAW	OBSERVATION
10mg/kg	0/3	0/3	0/3	No death
100mg/kg	0/3	0/3	0/3	No death
1000mg/kg	0/3	0/3	0/3	No death
750mg/kg	0/2	0/2	0/2	No death
1500mg/kg	0/2	0/2	0/2	No death
3000mg/kg	0/2	0/2	0/2	No death
6000mg/kg	½	½	0/2	Two deaths

LD50 =$\sqrt{A \times B}$

A = Maximum dose with 0% mortality

B = Minimum dose with 100% mortality.

Table 3: Comparison of the various enzymes levels between the negative control group and the test groups after 7days of treatment with the various plant extracts and ACT combined malaria therapy.

EXPERINMENT GROUP	AST Mean±SEM	P-VALUE	ALT Mean±SEM	P-VALUE	ALP Mean±SEM	P-VALUE
GROUP 2	172.00±0.95		42.80±0.86		452.80±2.24	
GROUP 3 (LG)	94.20±0.80	0.000*	28.80±0.49	0.000*	194.80±0.37	0.000*
GROUP 4 (PL)	141.6±7.10	0.000*	26.80±2.31	0.000*	204.40±1.12	0.000*
GROUP 5 (N)	173.20±1.62	0.850	24.20±1.28	0.000*	208.40±1.89	0.000*
GROUP 6 (N/PL)	160.80±9.72	0.082	23.40±0.93	0.000*	178.00±8.04	0.000*
GROUP 7 (N/LG))	127.00±2.17	0.000*	21.20±0.86	0.000*	167.40±2.38	0.000*
GROUP 8 (N/LG/PL)	124.80±5.63	0.000*	26.80±0.86	0.000*	157.20±1.39	0.000*
GROUP 9(PL/LG)	115.00±5.30	0.000*	24.40±0.81	0.000*	145.80±6.36	0.000*
GROUP 10(ATL)	129.60±1.44	0.000*	25.20±1.02	0.000*	156.00±5.63	0.000*
GTROUP 11(DAP)	137.20±0.86	0.000*	29.80±1.71	0.000*	160.80±2.94	0.00*

* The values of p<0.05 is considered as significant for differences in means when compared with group 2.
LG= Lemon grass, PL= Pawpaw leaf, N= Neem, ATL= artemetger/lumefantrine,
DAP= Dihydroartemisine/piperaquine phosphate.

From the table above, it is observed that the test groups showed significant decrease in the ALT and ALP levels when compared with the negative control group. There was also significant decrease in the AST level of all the test groups when compared with the negative control group except group 5

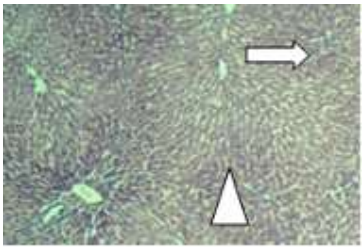
Table 4: Comparison of the various enzymes levels between negative control group and the test groups 14 days after treatment with the various plant extracts and ACT combined malaria therapy.

EXPERIMENT GROUP	AST Mean±SEM	P-VALUE	ALT Mean±SEM	P-VALUE	ALP Mean±SEM	P-VALUE
GROUP 2	100.00±6.32		68.80±1.16		465.20±4.53	
GROUP 3 (LG)	93.60±0.51	0.000	19.80±1.59	0.000	152.00±2.43	0.000
GROUP 4 (PL)	58.80±0.37	0.000	15.60±0.24	0.000	138.00±0.55	0.000
GROUP 5 (N)	58.00±2.43	0.850	15.40±0.93	0.000	132.80±1.93	0.000
GROUP 6 (N/PL)	71.40±1.69	0.082	19.80±0.86	0.000	142.60±2.56	0.000
GROUP 7 (N/LG)	67.60±3.19	0.000	18.80±1.07	0.000	143.80±1.69	0.000
GROUP 8 (N/LG/PL)	76.40±3.88	0.000	19.80±0.86	0.000	142.60±2.29	0.000
GROUP 9 (PL/LG)	60.40±1.33	0.000	18.40±0.68	0.000	132.00±1.55	0.000
GROUP 10 (ATL)	55.60±0.51	0.000	22.60±0.51	0.000	148.20±2.62	0.000
GROUP 11 (DAP)	58.40±0.68	0.000	24.60±0.87	0.000	152.80±0.97	0.000

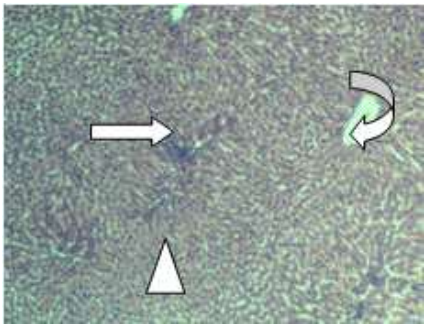
* The values of p<0.05 is considered as significant for differences in means when compared with group 2.
LG= Lemon grass, PL= Pawpaw leaf, N= Neem, ATL= artemetger/lumefantrine,
DAP= Dihydroartemisine/piperaquine phosphate

All the test groups showed significant decrease in the AST, ALT and ALP levels except the AST level of groups 5 and 6 which showed no significant difference when compared with group 2.

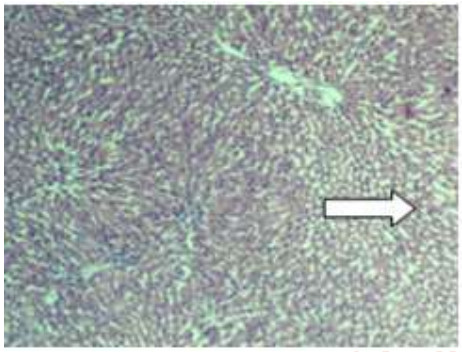
The histological assessment of the liver tissue gave an in-depth information on the protective activities of liver enzymes in ensuring organ recovery and maintaining the functionality of the liver. There were no visible lesions in the positive control group as well as the negative control, lemon grass, pawpaw leaf, neem, neem and lemon grass and dihydroartemisinine/piperaquine groups respectively and their hepatocytes were uncompromised. The group treated with neem and pawpaw leaf (6) showed an area of mild lymphocytic infiltration. The group with all three extracts (8) andthe artemether/lumefantrine group (10) showed moderately hypertrophied central vein with mild fluid exudation. Group 9 (pawpaw leaf and lemon grass) showed early signs of lipid infiltration on the liver tissue.



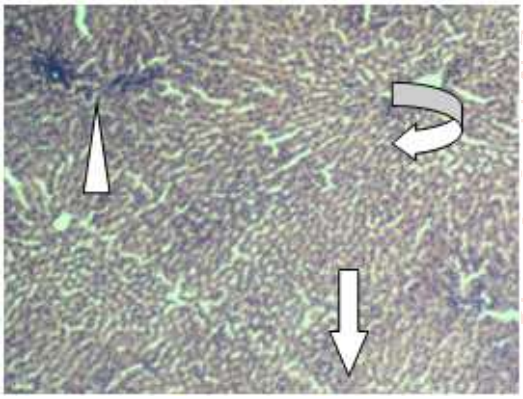
Group 3: Photomicrograph of liver tissue showing morphology consistent with healthy liver histology. (H&E, X100).



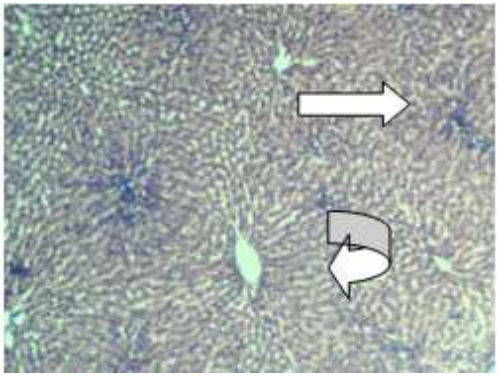
Group 4: Photomicrograph of liver tissue showing morphology consistent with healthy liver histology. (H&E, X100)



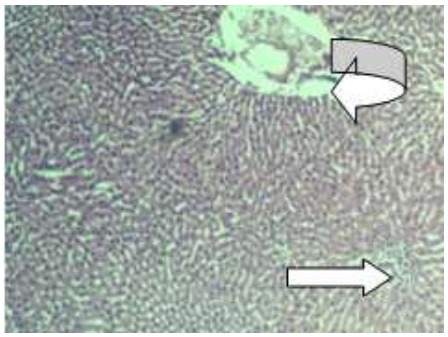
Group 5: Photomicrograph of liver tissue showing morphology consistent with healthy liver histology. (H&E, X100)



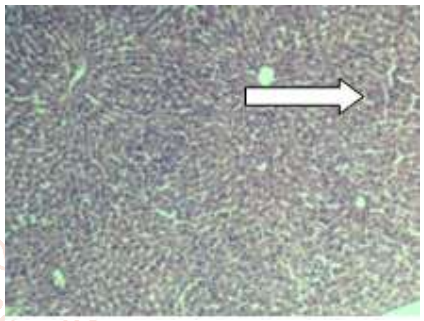
Group 6: Photomicrograph of liver tissue showing normal hepatocytes, with a focal area of mild lymphocytic infiltration (H&E, X100).



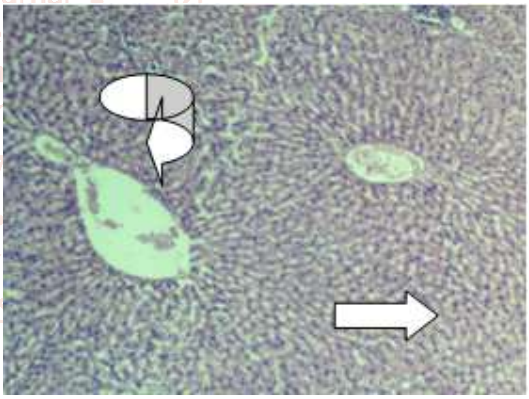
Group 7: Photomicrograph of liver tissue showing morphology consistent with healthy liver histology. (H&E, X100).



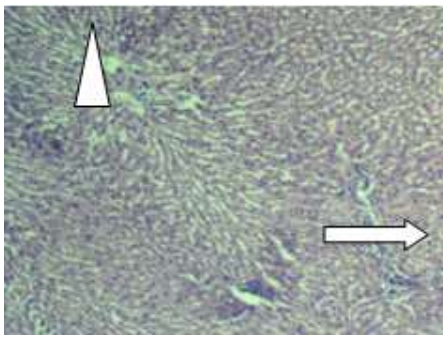
Group 8: Photomicrograph of liver tissue showing moderately hypertrophied central vein with mild fluid exudation and normal hepatocytes (H&E, X100)



Group 9: Photomicrograph of liver tissue showing normal liver cells but with early sign of lipid infiltration (H&E, X100).



Group 10: Photomicrograph of liver tissue showing moderately hypertrophied central vein with mild fluid exudation and normal hepatocytes (H&E, X100).



Group 11: Photomicrograph of liver tissue showing morphology consistent with healthy liver histology. The sinusoid and hepatocytes are normal with no obvious sign of injury (H&E, X100).

DISCUSSION

The enhanced activities of these serum marker enzymes observed in all the test groups in the present study correspond to the extensive liver damage induced by the parasite. The present study observed that the ALT levels in all the test groups increase significantly except in the group treated with neem+lemon grass. The ALP levels of the groups that was treated with NEEM+LEMON GRASS, NEEM+LEMON GRASS+PAWPAW LEAF and PAWPAW LEAF+LEMON GRASS respectively increasedwhen compared to the single extract group. This observation indicates that combination of the various extracts were not as toxic to the liver than the individual extracts, this suggests that in the treatment of malaria it is better to use the plants combination rather than using the individual plants..

In this present study all the test groups showed significant decrease in the ALT and ALP levels when compared to the negative control group. This indicates all the extracts used in the study have the tendency of reducing the toxicity introduced to the liver by the presence of the malaria parasite. There was significant decrease in the AST level of all the test groups when compared with the negative control group except the group that was treated with NEEM+PAWPAW LEAF extract, this is suggesting that a combination of Neem and Pawpaw leaf is a good herbal combination for effective functioning of the liver.

There was significant decrease in the AST level of the group that was treated with LEMON GRASS and PAWPAW LEAF+LEMON GRASS respectively when compared with the ARTHEMETER+LUMENFANTRINE group. This is indicating that the standard ACT (ARTHEMETER+LUMENFANTRINE) malaria drug is more toxic to the liver than the combination of PAWPAW LEAF and LEMON GRASS. Fourteen days after treatment with the various extract, all the test groups showed significant increase in AST and ALP level when compared to the control group, this indicates that fourteen days after treatment the effect of the various extract is still evident on the experimental rats.The non-significant difference between the ARTHEMETER+LUMENFANTRINE and DIHYDROARTEMISINE+ PIPERAQUINE group might be indicating that the effects of the both drugs elapsed at the same time.

Fourteen days after administration significant increase occurred in the AST level of the group that was treated with NEEM+PAWPAW LEAF,NEEM and NEEM+LEMON GRASS+PAWPAW LEAF extracts when compared with the group that was treated with ARTHEMETER+LUMENFANTRINE, this is suggesting that the detoxification activities of ARTHEMETER+LUMENFANTRINE to the liver stops before that of the plants.

High levels of ALP in serum indicate liver damage which is similar to this present study where, the increase in ALP activity in rats treated with LEMON GRASS extract and DIHYDROARTEMISINE+ PIPERAQUINE when compared with the negative control group, shows the liver damage, as a result of metabolic changes such as administration of toxin, liver cirrhosis, hepatitis, and cancer of the liver (*Trichopoulos and Willett, 1996*). Thus, it can be used as markers to estimate the extent of liver damage by the parasite.

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