Liver Function Status of Chloroquine Sensitive Plasmodium Berghei Nk65 Strain Passaged Mice Treated with Methanolic Extract of Moringa Oleifera Stem Bark

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ABSTRACT

The present study aimed at investigating the antiplasmodial activity of Methanol extract of Moringa oleifera stem bark as well as its effect on the serum levels of ALP, ALT, AST Total protein, Albumin and Liver histopathology. The mice were passaged with Plasmodium berghei NK65 and the antiplasmodial activity as percentage suppression obtained through a four (4) day suppression method. Total of forty mice randomised into eight groups (N= 5) were used for this studies. The group one and two were administered with distilled water and 1.5mg/kg artemether and 9mg/kg lumefantrine respectively. Group three, four, and five were treated with different doses of the extract while group six, seven, and eight were treated with the extract in combination with artemether-lumefantrine. The results obtained showed that 100mg/kg dose of the extracts gave the most significant suppression of malaria parasite at P < 0.05 even when compared with the artemether-lumefantrine and other groups. This signified that the extract has antiplasmodial activity which is not dose dependent. Co-administration of the standard drug with the various dose of the extract did not optimize the antiplasmodial of both agents as expected. For the activity of serum albumin, total protein, ALT, AST, ALP and liver histopathology tests compared with the control suggest no hepatotoxicity. It was concluded that the extract has antiplasmodial activity at low dose and has no observable hepatotoxicity.

Keywords: Alanine transaminase, Aspartate transaminase, alkaline phosphatase, Malaria, Liver histology, Albumin

INTRODUCTION

Malaria is a global disease that is predominant in the tropics and caused by blood parasites (a type of single cell microorganism of the plasmodium). Malaria symptoms typically include fever, fatigue, vomiting and headaches. In severe cases, it can cause yellow skin, seizures, coma or death (Caraballo, 2014). These symptoms usually begin ten (10) to fifteen (15) days after being infected. In those who have not been properly treated, disease may recur months later. In those who have recently survived an infection, re-infection typically causes milder symptoms. This partial resistance disappears over months to years if there is no ongoing exposure to malaria (Caraballo, 2014).

Commonly, the disease is transmitted by the bite of an infected female anopheles mosquito. This bite introduces the parasites from the mosquito’s saliva into the person’s blood. The parasite then travels to the liver where they mature and reproduce. The parasite has five species which are the Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae, Plasmodium vivax and Plasmodium knowlesi. The species, P. knowlesi rarely causes disease in humans. In Nigeria, malaria is mostly caused by P. falciparum and P. malariae. Malaria has a great morbidity and mortality than any other infectious diseases of the world (White, 2004). The disease is widespread in tropical and sub-tropical regions which are present in a broad bend around the equator (Caraballo, 2014). This includes much of sub-Saharan Africa, Asia and Latin America. The World Health Organization estimates that in 2012, there were 207 million cases of malaria. That year, the disease is estimated to have killed between 473,000 and 789,000 people, many of whom were children in Africa. The types of drugs and the length of
treatment will vary depending on; which type of malaria parasite has caused the infection, the severity of symptoms, age pregnancy

The most common antimalaria drugs include, Chloroquine, Quinine sulfate (Quilaquin), hydroxyl-chloroquine (Plaquenil), Mefloquine, combination of atovaquone and proquani (Malarone). The history of antimalaria medicines has been marked by constant struggle between evolving drug resistant parasites and the search for new drug formulations. In many parts of the world, for instance, resistance to chloroquine has rendered the drug ineffective. This study was aimed at determine the antiplasmodial activity by percentage parasitemia suppression and the effect of methanolic extracts of Moringa oleifera stem bark on the liver of plasmodium infected mice.

JUSTIFICATION OF THE STUDY

The justification lies on the relative cheapness, abundance and safety use of Moringa oleifera stem bark extract for the treatment of malaria.

MATERIALS & METHODS

Collection of Plant Material

The plant material Moringa oleifera stem bark used for this study was collected from Moringa oleifera tree at medical centre premises in Akanu Ibiam Federal Polytechnic Unwana Afikpo in Ebonyi State and authenticated by a taxonomist.

Extraction of Plant Material

The freshly obtained Moringa oleifera stem bark was air dried for one month. It was grinded with a mechanical blender to fine particles, 48g of the Moringa oleifera stem bark was soaked in 800ml methanol for 2 days; it was sieved with cheese cloth and filtered with a filter paper. It was poured in a clean beaker, covered with a net and left to cold dry under the fan for two weeks in order to obtain the crude extract.

Reconstitution of the Extract

Twenty (2g) of the crude extract of the Moringa oleifera was dissolved in distilled water to a known dose of 50mg/ml.

Sources of the Animals

Forty inbred albino mice were obtained from the animal house of the department of veterinary medicine, University of Nigeria, Nsukka, were weighed and used for this study. The animals were kept in well ventilated rodent cubicles. The albino mice were kept for two weeks to acclimatize before passing them with the parasite (Plasmodium berghei).

Source of the Parasite

The unicellular microscopic animal, Plasmodium berghei Nk65 stains was sourced from the department of veterinary medicine, University of Nigeria Nsukka. The parasite was maintained by serial passaging in the donor mice.

Passaging of the Mice

The 4-Day Suppressive antimalarial tests in Animal Model were performed using P. berghei NK65 strain, maintained by serial weekly passages of infected blood in mice. Tests were performed as described by Peters (1965) with some modifications by Carvalho et al. 1991. Briefly, forty mice inoculated by intraperitoneal route with $1 \times 10^5$ infected red blood cells were kept together for 2 to 16 h.

Experimental Design/Treatment

A total of forty albino mice were used and weighed and also randomized into eight (8) groups of five mice each.

Group I: NCTRL; this group were given nothing except distilled water.

Group II: PCTRL, this group was treated with 1.5mg/kg artemether and 9mg/kg Lumefantrine

Group III: LDE: this group was treated with 100mg/kg dose of methanolic stem bark of Moringa oleifera extract.
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Group IV: MDE; this group was treated with 200mg/kg dose of methanolic stem bark of *Moringa oleifera* extract.

Group V: HDE; this group was treated with 300mg/kg dose of methanolic stem bark of *Moringa oleifera* extracts.

Group VI: COLDE; Co-administration of standard drug with lower dose. This group was treated with co-administration of standard drug and extracts at 100mg/kg dose.

Group VII: COMDE; Co-administration of standard drug with medium dose, the group was treated with co-administration of standard drug and extract at 200mg/kg dose.

Group VIII: (COHDE); Co-administration of standard drug with high dose; this group was treated with co-administration of standard drug and extract at 300mg/kg dose.

**Parasitemia Count**

The blood sample was picked from the mice tail blood and placed on a clean slide and a thin smear was made, the smear was fixed with methanol and stained with Giemsa stain for a few minutes and washed off under a running tap water and left to air dry for about 25 minutes and then viewed under a microscope at x400 magnification.

**Biochemical Analysis**

The animals were sacrificed through ocular puncture, with broken heparinised capillary tubes. The blood collected was allowed to clot for about 10 minutes, centrifuged at about 4000rpm for 5 minutes. The serum obtained was stored in a refrigerator at a temperature of about 4°C and below until use.

Serum Albumin and Total protein were determined by the colorimetric methods of Bradford was used as described by (Gowenlock et al., 1988). Determination of Serum Alanine Transaminase (ALT) and Aspartate Transaminase was done by colorimetric method of Reitman and Frankel (1957), and Alkaline Phosphatase (ALP) by Tietz (1976).

**Histopathological Study**

This is the microscopic examination of tissue in order to study the manifestations of disease. The animals were sacrificed and the liver extracted. The organ was fixed in 10% formalin. After complete fixation, the block was embedded in a paraffin and sections cut at 5m which was then stained with haematoxylin and eosin and mounted in Canada balsam. Microscopic examination of the sections was then carried out under a light microscope.

**Statistical Analysis**

All results obtained were statistically analyzed and expressed as percentages and as Mean ± SEM; and the significance of differences of the different drug treated groups were determined using ANOVA. Values of P < 0.05 were considered as significant.

**RESULT**

![Figure 1: Mean percentage parasitemia suppression](image)

*Figure 1. Mean percentage parasitemia suppression.*

*fig.1, Group 3 mice have significant decrease when (p<0.05) compared to the negative control (NCTRL), group 1 mice.*
Mean Serum Albumin
In fig 4.2, Group 6 mice have significant decrease when (p<0.05) compared to the negative control (NCTRL), group 1 mice.

Mean Serum Total Protein
In fig 4.2, Group 6 mice have significant decrease when (p<0.05) compared to the negative control (NCTRL), group 1 mice.

Alkaline Phosphatase (ALP)
In fig 4.3, There is significant decrease (p<0.05) in the ALP level of the mice treated with the extract at lower dose and at the significant increase (p>0.05) in the ALP level of treated mice with the extract at high dose when compared with the negative control. There is also a significant decrease (p<0.05) in the ALP level of the mice treated with co-administrations of the extract with artemether-lumefantrine at high dose when compared with the negative control.

Alanine aminotransferase (ALT)
In fig 4.3, There is significant decrease (p<0.05) in the ALT level of the mice treated with the extract at a medium dose when compared with the negative control. There is also a significant decrease (p<0.05) in the ALT level of the mice treated with co-administrations of the extract with artemether-lumefantrine at various doses.
HISTOMORPHOLOGICAL STUDIES

Sections of the liver were collected from animals of the various experimental groups, fixed in 10% phosphate buffered formalin for a minimum of 24 hours and subsequently prepared for histopathological examination using standard techniques. The histopathological examinations showed diverse changes in the hepatic histo-architecture, varying from degenerative to necrotic hepatocellular changes, hyperplasia of the kupffer cells, moderate to severe inflammatory cellular infiltration and intracellular or connective tissue haemazoin depositions.

Slide: Nctrl

A photomicrograph of the liver from group 1 showing a moderate to severe cellular infiltration of the portal area (arrow). Haemazoin pigments are visible in around the portal area (white arrow). Central vein (V); Portal area (P). H&Ex100; x400.

Higher magnification of a section of the liver from group 1 showing periportal infiltration of inflammatory leucocytes (black arrow) and haemazoin pigments (white arrow). Portal area (P). H&Ex400.

Slide: Pctr

A photomicrograph of the liver section from group 2 showing vacuolar degeneration of the centrilobular and midzonal hepatocytes {note the pale stained hepatocytes around the central vein (V) in contrast to the deeper stained hepatocytes around the portal area (P)}
Higher magnification of the liver from group 2 showing cytoplasmic vacuolations in the hepatocytes around the central vein (V). H&Ex400

Slide: Lde
A photomicrograph of the liver from group 3 showing vacuolar degeneration of the centrilobular to midzonal and periportal hepatocytes (note the pale staining characteristic). Mild cellular infiltration can be observed around the portal area (arrow). Central vein (V); Portal area (P). H&Ex100.

Slide: Mde
A photomicrograph of the liver from group 4 showing vacuolar degeneration and necrosis of the centrilobular to midzonal hepatocytes (white arrow) while the periportal hepatocytes appear normal (black arrow). Central vein (V), Portal area (P). H&EX100.
Sections of the liver from this group showed a severe vacuolar degeneration of the centrilobular hepatocytes and moderate to severe periportal infiltration of inflammatory leucocytes.

A photomicrograph of the liver showing hepatocellular vacuolar degeneration of the centrilobular hepatocytes {note the pale areas around the central vein (V)} and severe cellular infiltration (arrow) around the portal areas (P). H&Ex100.

Sections of the liver from animals in this group showed kupffer cell proliferation and a mild random, multifocal aggregations of inflammatory leucocytes (arrow). Central vein (V); Portal triad (P). H&Ex100
Slide: Comde
Sections of the liver from this group showed primarily an inflammatory process. There were widespread severe periportal infiltration of inflammatory leucocytes as well as kupffer cell proliferation.
A photomicrograph of the liver from group 7 showing severe cellular infiltration (arrow) around the portal area (P). Central vein (V). H&Ex100.

Slide: Cohde
Sections of the liver from animals in this group showed histopathological changes similar to those observed in group 7. There were severe periportal infiltration of inflammatory leucocytes and kupffer cell proliferation.
A photomicrograph of the liver from group 8 showing severe periportal infiltration of inflammatory leucocytes (arrow). Portal area (P); Central vein (V). H&Ex100.

DISCUSSION
Traditional cultures in various parts of the world have long used *Moringa oleifera* in their herbal medicine repertoire for ailments ranging from gout to various inflammations and fever. The *Moringa oleifera* stem bark is medicinal, it has anti-inflammatory activities to treat arthritis, rheumatism, and it encourages urination and can be used as relaxant for epilepsy. Every part of the *Moringa* tree has been used effectively against varying ailments (Mughal et al., 1999).
From fig 1 above, it could be seen that extracts at low dose of 100mg/kg body weight showed the highest suppression to the parasites even more than the control drug (artemether-Lumefantrine) at the dose administered. This reveals that the antiplasmodial activity of methanol extract of *Moringa oleifera* stem bark is not dose dependent. That is to say that it does not increase with increase in dose as one would ordinarily expect rather, the converse is the case.

For the co-administration groups, the same observations were made. But when the percentage suppression of the parasites in the co-administration groups were compared with the groups and given the various doses of the extracts and the standard control drug, it could be inferred that the co-administration controls the chemotherapeutic efficacies of both agents. This could be explained in the light of negative drug – drug interactions.

The total protein consists of albumin and globulin. The liver to a large extent synthesizes albumin and some fractions of globulin. The slight decrease in the serum albumin level observed from NCTRL group correlates with earlier reports by other workers (Adebayo et al., 2009; Adeosun et al., 2007 and Premeratria et al., 2001). This still indicates slight hepatic dysfunction since albumin is excessively synthesized in the liver. It also confirms that the decrease induction was perfect. The reduced serum albumin level causes reduced plasma osmotic pressure. In this same light, the slight increase observed with the control drug group and extract treated groups may suggest hepatotoxic effect.

The measurement of the activities of enzymes in the tissue and body fluids plays a paramount and will known the role in disease investigation and diagnosis (Malomo, 2000). These enzymes such as phosphatase, dehydrogenase and transferase, get into the blood through linkage from disrupted cell membranes in damaged tissues (Adaramoye et al., 2008).

ALP has been reported to be a marker enzyme for plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974). ALT activity in the plasma is a more specific indicator of liver damage affecting cell integrity. ALT and ALP activities, at all doses administered of the extract and its co-administration with artemether-lumenfantrine, were not significantly changed in plasma, suggesting that they may not interfered with plasma membrane integrity of the hepatocytes and other metabolic activities mediated by ALP in the liver. The lack of change in plasma ALP activity also suggests that the extract and its co-administration does not cause hepatobiliary obstruction (Panteghini and Bais, 2008).

**HISTOPATHOLOGY**

*Plasmodium* is known to cause degenerative and inflammatory changes in the liver. The inflammatory reaction is due to a direct response to the presence of the parasite in the liver while the degenerative change is a secondary response to the effect of the parasite on the blood cells which results in decreased oxygen availability to the hepatocytes. Both reactions were observed in this study. NCTRL showed a typical malarial hepatitis with deposition of haemoglobin pigment. This confirmed that the infection was successful.

PCTRL showed primarily, a degenerative change inflammatory changes were not apparent. This suggests that the drug was able to prevent malarial hepatitis. However, the observed degenerative change could be due to the effect of the parasites already inside the red blood cells as at the time of drug administration.

In LDE and MDE, centrilobular to midzonal and occasionally, perportal degeneration of the hepatocytes and a mild inflammatory change were observed. This suggests that the test extract at 100mg/kg and 200mg/kg was protective against malarial hepatitis but not against the hepatopathy (degenerative change in the liver) induced by parasitized red blood cells.

In HDE, a moderate to severe inflammation was observed as well as degenerative changes. This could mean that high doses of this extract are not protective against malarial hepatitis or malarial hepatopathy.

In COLDE, mild inflammatory changes which can be considered insignificant seen. This suggests that the combination was protective against malarial hepatitis and hepatopathy.

The histopathological examination in COMDE AND COHDE groups showed severe hepatitis. This implied that the combination of the extracts and standard drugs failed to protect against malarial hepatitis. There could have been drug-extracts interaction at the doses administered that neutralized the agents and consequent led to loss of desired activity against malaria parasites.
CONCLUSION

Moringa oleifera stem bark has antiplasmodial activity and does not show hepatoprotective and hepatotoxic properties at the dose administered. The combined administration of Moringa oleifera stem bark extract with artemether lumefantrin as malaria therapy is ineffective and therefore does not provide tangible efficacy.

More research is also recommended to isolate the bioactive component of the extract responsible for its antimalaria activity and offer more scientific explanation why co-administration here does not optimize efficacy.

REFERENCES


