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ORIGINAL ARTICLE

Antioxidants Level of Chloroquine Sensitive *Plasmodium berghei* Nk65 Strains Passaged Mice Treated with Crude Mathanolic Extract of *Moringa oleifera* Stem Bark

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ABSTRACT
This present study aimed at investigating the antioxidant effect of methanolic extract of Moringa oleifera stem bark on Plasmodium berghei passaged mice. The 4 day malaria suppressive method was used to determine antimalaria effect and colorimetric method employed for determination of serum levels of MDA, GSH, Vit E, SOD, and GPx. The result obtained for antimalaria test showed suppression presented that of negative control (NCTRL) as 53% with no significance except for that of extract low dose at 100mg/Kg (LDE) which is30%. For antioxidants, SOD showed no significance in the groups; GPx activities was significantly low at 200mg/kg (0.4IU/l), 300mg/Kg (0.4IU/L) and co-administration with 300mg/Kg of the extract but significant increase at 100mg/Kg (1.3IU/L) and co-administration with 200mg/Kg of the extract (2.7IU/L). The serum levels of Vit. E and GSH in all the groups showed no significance. MDA showed slight decrease without significance in all the groups except at 200mg/Kg. and co-administration of the standard drug and the extract 100mg/Kg where there was an increase with significance. It was concluded after considering the assayed enzymic anti-oxidants and non enzymic anti-oxidants to had anti-oxidant effect which is beneficial in handling malaria cases and other diseases in general.
Keywords: Chloroquine, Plasmodium berghei, Moringa oleifera Stem Bark

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INTRODUCTION

Malaria is one of the most infectious diseases responsible for the high rate of mortality in developing countries. Malaria is a picoplexan protozoa parasite which belongs to the genus plasmodium [5], and is known to contain a picoplast which is an indispensable organelle for the parasite metabolic process. Four species of plasmodium parasite are known to cause infection in humans; *Plasmodium Falciparum, Plasmodium Vivax Plasmodium Ovale* and *Plasmodium Malarieae Plasmodium Knowlesi*, previously known to infect monkeys have been implicated in human infections as well.

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reaction can produce free radical, in turn; these radicals can start chain reactions. When the chain reactions occur in a cell, it can cause damage or death of the cell. These antioxidants terminate these chain reactions by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acids or polyphenols [3].

We have two categories of antioxidants which are enzymic and non-enzymic antioxidant the non-enzymic ones include, glutathione, vitamin C, vitamin A, and vitamin E, and the enzymic ones include, Catalase, superoxide dismutase and various peroxidase. Insufficiencies or inhibition of antioxidant enzymes causes oxidative stress which may damage or kill cells.

Evaluation of various plant products according to their traditional uses an medicinal value is based on their therapeutic efficacy, which lead to the discovery of newer drugs from various plant sources, one of such medicinal plant is *Moringa Oleifera* which is one of the most cultivated species of the family Moringaceae, which is commonly known as sahajan in hindi, horse radish in English. *Moringa Oleifera* is a fast growing ever green tree that usually grows up to 10 – 12 meters in height and distributed in sub Himalayan trails, Assam, Bengal and Peninsula India. Various pharmacological properties are attributed to it such as antiplasmodic, diuretic, expectorate and abortifacient. Every part of Moringa Oliefera is said

to have a beneficial properties that can serve humanity so the whole parts of the plant has a very high medicinal importance as a non-food product [2].

This study focused on the determination of antioxidants effect on plasmodium passage albino mice treated with methanol extracts of *Moringa Oleifera* stem bark.

MATERIALS AND METHOD

PLANT MATERIAL

The stem bark of *Moringa Oleifera* used in this project research was collected from *Moringa Oleifera* trees within Akanu Ibiam Federal Polytechnic Unwana premises in Ebonyi state, Nigeria..

SOURCES OF THE PARASITE AND ANIMAL

The chloroquine sensitive *Plasmodium berghei* NK65 strains used in this work was sourced from the department of veterinary medicine university of Nigeria Nssuka. A total of 40 albino mice were gotten from poultry around the localities of the University of Nigeria Nssuka which was maintained by serial passage of the plasmodium.

EXTRACTION OF THE CRUDE EXTRACT

The tree stem bark extract was obtained by soaking 500g of blended stem bark in 1000ml methanol for two days, after which it was sieved with a cheese cloth and then, filtered with a filter paper, poured in a beaker and dried to a constant weight solid.

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 [17] with some modifications by (16). Briefly, forty mice inoculated by intraperitoneal route with 1×10^5 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.

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 25minutes and then viewed under a microscope at x400 magnification.

BIOCHEMICAL ANALYSIS

The animals were sacrificed through ocular puncture with broken heparinised capillary tubes and the blood sample was collected with an heparin sample bottle which was then centrifuged at about 3000rpm for 10 minutes after which the serum was collected using Pasteur pipette and stored in the refrigerator at a temperature of about 4^oC. The plasma obtained was used to determine the following parameters.

The serum glutathione peroxidase and Superoxide dismutase activity was assayed by the method of Arthur and Boyne [1]. Vitamin E content was determined by the method of [15]. The glutathione level was determined by the method of [14].

Statistical Analysis

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

RESULT

The mean value of the glutathione and superoxide dismutase (SOD) results for each group was taken and used to make analytical chart showing the bar chart representing each of the group.

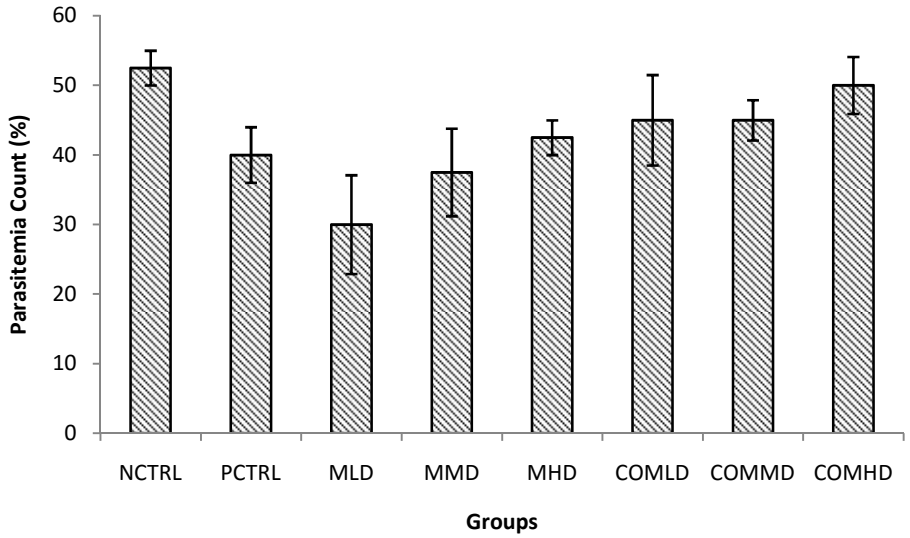

Fig 1: Effect of *Moringa oleifera* and Artemether-Lumenfantrin on malaria parasitized albino mice

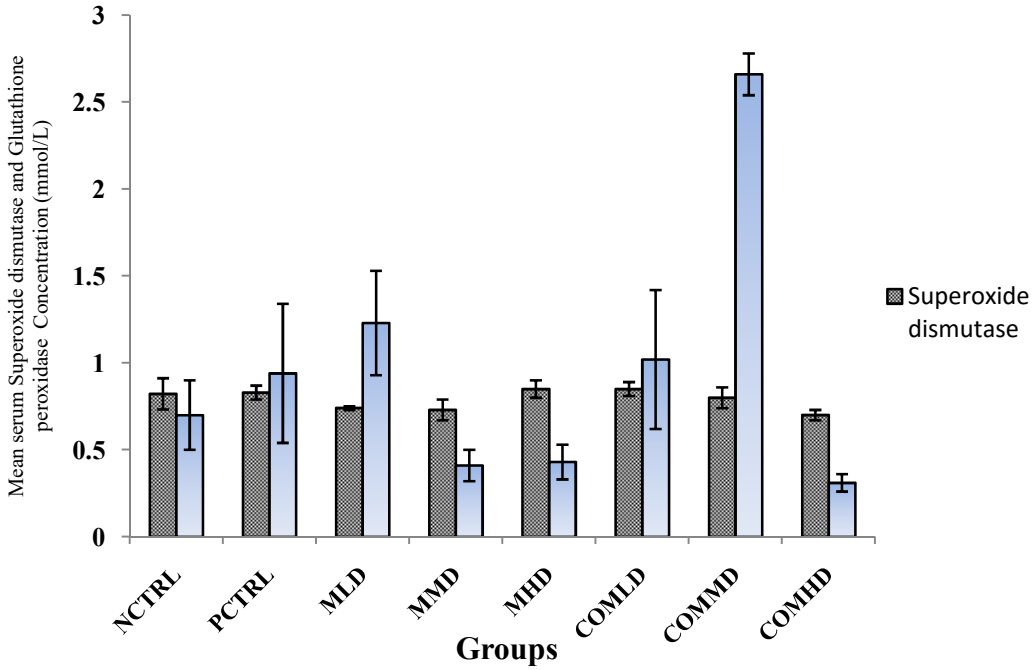

Fig 2: Effect of administration of methanol extract of *Moringa oleifera* stem bark on superoxide dismutase and Glutathione concentration of albino mice.

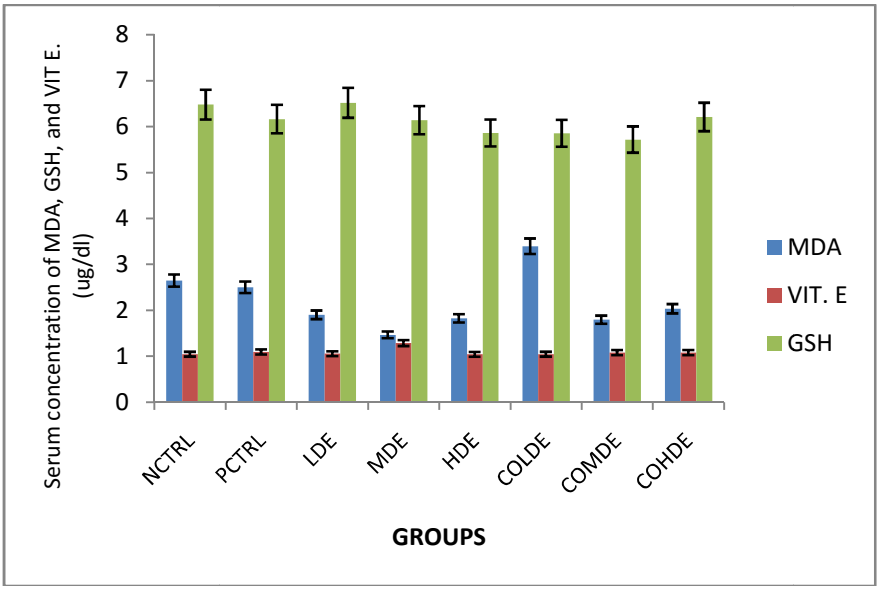

Fig 4.2: Effects of doses of Artemether-Lumefantrine and methanolic extract of *Moringa oleifera* stem bark on on Glutathione (GSH), Vitamin E (Vit E) and Maloaldehye (MDA). (each value is a mean of 4 determinant).

INTERPRETATION

In fig 3 above, FOR MDA

There is a significant decrease ($p<0.05$) in the level of MDA of mice treated with extract of *Moringa oleifera* stem bark at MLD, MMD and MHD when compared with the negative control and also there is significant increase ($p<0.05$) in MDA level of mice treated with co-administration of the extract with artemether lumefantrine at lower dose when compared with the negative control. Co-administration of extract with artemether lumefantrine at COMMD showed a significant decrease in MDA level.

FOR VITAMIN E.

There is no significant difference ($p>0.05$) in Vit E. level mice treated with extract of *Moringa oleifera* stem bark at various doses and the co-administration of the extract with artemether lumefantrine at various doses when compared with negative control.

FOR GSH

In GSH, there is no significant different ($p>0.05$) in glutathione level of mice treated with extract at various doses and co-administration of the extract with Artemether lumenfantrine at various doses when compared with the negative control.

DISCUSION

For hundreds of years, traditional healers have prescribed different part of *Moringa oleifera* for treatment of skin disease, malaria, respiratory illnesses, ear and dental infections, hypertension, diabetes, cancer treatment, and water purification etc and they have promoted its use as a nutrient dense food source [9]. Biomarker of oxidative stress reflect environmental pro-oxidant and antioxidant and also serve as a surrogate measure of a disease process [7]. The protective effect of methanolic extract of *Moringa oleifera* stems bark on erythrocyte GSH, Vit. E and MDA concentration may be attributed to the presence of phytoconstituents (Polyphenols, tannins authocyanin, glycosides, thiocarbamates) that scavenge free radical, activate the antioxidant enzymes, and inhibit oxidases [7]. Glutathione (GSH) acts as an antioxidant both intracellulary and extracellulary, and it is a major non protein sulfhydrl compound, with many biological functions including maintenance of membrane protein-SH groups in the induced form, the oxidation of which can otherwise cause altered cellular structure and function (9). Membrane – SH group oxidative damage may be an important molecular mechanism inducing changes in the membrane micro-elasticity or whole cell deformability of erythrocytes and conditions of physiological and pathological oxidative stress. The methanolic extract of *Moringa oleifera* stem bark contains certain non phenolic, biologically active components such as selenium, thiocarbamates, glucosinolates, its hydrolysis products as glucoraphanin, isothiocyanate sulforaphane, nitriles [8]. In addition to the phenolics, which could serve as various reactive oxygen species free radical under *in-vivo* condition.

The result obtained in the study for both the extract groups and the extract co-administration with the standard drug groups doses not show any significant reaction in the serum levels of GSH, and Vit E. the serum levels of these non enzymic antioxidants suggest that the extract in both categorized does not interfere with the synthesis and degradation of these antioxidants. This also suggests protection against oxidative stress and its consequences.

Like GSH content, a similar pattern was observed for MDA, methanolic extract of *Moringa oleifera* stem bark maintained the normal level of erythrocyte MDA suggesting that the extract may have a mixture of biomolecules with hydroxyl groups that prevent the abstraction of hydrogen atom from the double bond of lipid bilayer thereby avoiding the damage of lipid membrane. Our observations are in agreement with the previous findings of [13], where high content of phenolics in the methanolic extract of leaves compared to aqueous extract was reported.

Recently, we have showed concentration dependent hydroxyl radical scavenging ability of *Moringa oleifera* stem bark extract in deoxy-ribose degradation assay [9] and our present findings may impart not only suggest the use of M. stem bark, leaves an fruit as a supplementary/dietary antioxidant in nutraceutical and cosmoceutical, but also improve the ethnopharmacological knowledge of M. plant, which paves the way for use of fruit, stem bark and leaves as an economically viable source of natural and potent antioxidant.

The antimalaria chemotherapy is the keystone of malaria control effort. But the rate of resistance developed by these malaria parasites has become a serious problem in the use of antimalaria drugs [11]. The use of herbal drugs in the control of malaria is now confirmed to be more affordable and safer. In this study, the antiplasmodial efficacy of methanol extract of *Moringa Oleifera* stems bark has been evaluated in vivo.

It was also observed that both agents acts better as malaria chemotherapeutic agents when administered single than in co-administration. This suggests from the biochemical view that co-administration councils the antiplasmodic activity of each agent possibly by depurative drug-drug interaction. The neutralization of antimalaria activity of co-administration seems to depend on the dose of the extract which increases with the dose increment.

Reactive oxygen species has been reported to play an important role in malaria infection pathology [6]. To back up this claim increase in xanthine oxidase and lipid peroxidation was reported in plasmodium berghei infected mice [6]. Although, reactive oxygen species can be deleterious to tissues and organs, several studies have equally shown that increased generation of free radicals occasioned by P. berghei is beneficial to host in combating against intra-erythocytic parasite growth. This was employed in pro-oxidant treatment of malaria infection. Although pro-oxidant (artemisinin and its derivatives) treatment is effective, an antioxidant drug for malaria treatment can be more effective and tolerated by the system [4]. The result of the study recorded that extract administered has some antioxidant potentials as it was able to bring down the serum level of glutathione peroxidase (GPx) and superoxide dismutase (SOD) below the untreated control and the standard drug control as shown in fig. 4.2 at medium and high doses.

For the co-administration groups, the serum level of GPx and SOD were slightly above those of single extract administered group, but decreases as the dose of the extract in the co-administration increases. This implies that the antioxidant effect of the extract nullified the pro-oxidant activity of the standard drug to offer more protection to antioxidative stress and its consequences.

CONCLUSION

In conclusion, methanol extract of *Moringa Oleifera* stem bark has its peak antiplasmodial activity at the low dose (100mg/kg). The co-administration of the extract and standard drug (Artemether lumefantrine) as observed in the study did not optimize or potentiate the antiplasmodial activity of both agents and should be discouraged. It also has an antioxidant effect which presented itself in a dose dependent manner. However, further studies are hereby recommended to isolate the bioactive component of the extract that is responsible for the observed antiplasmodial and antioxidant activities observed.

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