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Antiplasmodial and Toxicological Effects of Ethanolic Extracts of *Moringaoleifera* and *Annonamuricata* Leaves on Albino Rats

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Abstract: ***Malaria is a disease caused by Plasmodia species. In a bid to address the health problems posed by malaria especially in tropical regions, plant based products are often being resorted to. This study was aimed at evaluating the anti-plasmodial and toxicological effects of Moringaoleifera and Annonamuricataethanolic leaf extracts in albino rats. In this study, the experimental animals were all (except for the normal control) inoculated with Plasmodium berghei infected erythrocytes and were treated with the plant extracts and reference drug (combisunate). The suppressive ability and Biochemical indices were carried out after five days of treatment. The results showed that there was reduction in the parasitemia level of the groups treated with Annonamuricata relative to the untreated control which was significant at p<0.05. Except for the groups treated with Moringaoleifera leaves. The biochemical tests revealed a decrease in hepatic and renal integrity markers of Annonamuricatatreated groups while the reverse is seen in Moringaoleifera treated groups and the normal control. Hence, it can be inferred that Moringaoleifera has low or no antiplasmodial effect, whereas Annonamuricatashowed moderate antiplasmodial activity against P. berghei and can serve as a base for new anti-malaria drug.***

Keywords: Toxicological, antiplasmodial, *Moringaoleifera, Annonamuricata, Plasmodiumberghei*

1. Introduction

The usefulness of herbal products is of global importance due to their less side effects, accessibility and inexpensiveness when compared with conventional orthodox drugs [1]. In fact, plant based medicines have now been considered as another source of therapy for various sicknesses and diseases. This is because; plants have been found to be safer with little or no side effects because of the presence of natural ingredients known as secondary metabolites[2]. In most rural settlements in Africa, primary health care services remain dependent on the use of several medicinal plants since synthetic drugs have proven to be expensive, unavailable and side-effect loaded [3]. In the same thought, studies on the efficacies of these tropical plants as new sources of natural, effective, cheaper and potentially less toxic drugs has not been completely explored [4],[5]. As a result of the increasing resistance of malaria parasites to chloroquine, a cheap and commonly used drug for malaria in Nigeria, Artemisinin, an herbal therapy, indigenous to China, based on active principle of italiaannua, has been introduced into the Nigerian market. However not much has been done to promote antimalaria properties of indigenous medicinal plants [6].

Moringaoleifera is a well-known and an important plant source as it contains secondary metabolites and is used by so many countries around the world due to its multipurpose medicinal and nutritional properties. Extracts from the plant are believed to have antitumor, antipyretic, antiepileptic, antioxidant, antibacterial, antifungal, diuretic and antihypertensive activities[7]. Extracts of *M. oleifera* seeds have shown marked potential against the larvae, pupae and adult insect stages of mosquitoes. Extract of *M. oleifera* have been shown to contain phytochemicals such as glycosides, flavonoids, steroids, terpenoids, saponins, tannins and anthraquinones [8],[9]. The potency of different solvent extracts of various parts of this plant against *P. berghei* has not been comprehensively investigated. The aim of this study was to assess the *in vivo*activity of ethanolic extract of *M. oleifera* against the *P. berghei* parasite.

Annonamuricata extract presents antioxidant, anti-inflammation, antimicrobials, antiparasitic, antidiabetes, antihyperlipidemia, hepatoprotective, and anticancer activities [10],[11].They phytochemical evaluation of *A. muricata* extracts have shown the presence of various compounds such as alkaloids, polyphenols, flavonoids, essential oils, cyclopeptides, and kaempferol. In addition, *A. muricata* is known to be a rich source of acetogenin compounds as the active compounds in this plant extracts [12]. Furthermore, the leaf extracts of *A. muricata* was assayed against *P. falciparum* and showed promising antimalarial effect [13]. However, antimalarial activity of this plant ethanolic extract against *P. berghei* infected rat model has not yet been reported. Hence, the present study aimed at evaluating the *in vivo* antimalarial activity of *A. muricata* ethanolic leaf extract against *P. berghei* infected rats.

1.2 Aim and Objectives of the Study

This study is aimed at evaluating the anti-parasitic and toxicological properties of *Moringaoleifera, and Annonamuricata*ethanolic leaf extracts in albino rats.

1.2.1 Objective

In this study, the following objectives were set to be accomplished:

a) To determine the plasmodium suppressive effect of the extracts in albino rats

b) To determine the toxicological effects of the extracts on rats by assessment of biochemical parameters.
c) To evaluate the effect of dose variation of each extract on the anti-plasmodial potency and their toxicological effects in albino rats.

1.3 Significance of the Study

People leaving in the rural areas in most underdeveloped countries of the world just as the case are in Nigeria age faster than their peers in the city. The burden of malaria makes a serious contribution to the reduction in the economy of most countries. This is most felt in rural areas of sub-Saharan Africa where poverty and deprivation are prevalent. The availability of natural products like medicinal plants will greatly help to solve the healthcare problems of these rural communities. Getting a leaf that can handle the problem of malaria and stress accumulation will be of great importance knowing well that the rural area have abundant herbal plants when compared to the cities.

2. Materials and Methods

2.1 Source and Identification of Plant Material

Fresh leaves of *Moringaoleifera* and *Annonamuricata* leaves were collected from the University of Port Harcourt Botanical Garden Plant science and biotechnology farm (PSB), University of Port Harcourt in Obio/Akpo Local Government Area, Rivers State, Nigeria. The plant sample was identified and authenticated at the herbarium unit of the Department of Plant Science and Technology (PSB), University of Port Harcourt.

2.2 Plant Extract Preparation

The leaves of *Moringaoleifera* and *Annonamuricata*were obtained fresh and were sorted out and washed under running tap water to remove dirty. Thereafter, the leaves were also air dried under shade. They were later ground into fine powder using an electrical blender. The two grounded fine powder were kept in tightly closed brown bottle until used for extraction. The powders (0.5 kg) were placed separately in a thimble of a soxlet extractor apparatus where they were extracted using absolute ethanol for 72 hours. The filtrates were then filtered using a filter paper and the filtrates were condensed in a rotary evaporator. A greenish residue weighing 43.45, 30.56 and 17.01g respectively were obtained. The extracts were kept in air tight sample bottles in a refrigerator until needed.

2.3 Experimental Design

The method used to evaluate the anti-malarial effect of *Moringaoleifera* and *Annonamuricata*leaves was that described by Ryley and Peters [14] with slight modification. They animals were acclimatized for several days before use. They were housed in a specially designed plastic/wire gauze animal cage and were placed on standard feed and given access to water *ad libitum*. Fifty two (52) albino rats were selected and forty eight (48) of these were injected intraperitoneally with 0.5ml of blood infected with 2×107/ml *Plasmodium berghei* (NK65 strain) on the first day. The inoculation of the parasites into the animals was done at the malaria research laboratory, centre for malaria research and phytomedicine University of Port Harcourt. The infection was confirmed by viewing the Giemsa – stain blood smear obtained from the tail of the infected rats and studied under the microscope. This was established on the fourth day. After the confirmation, the animals were put into eighteen groups of four rats per group. Group 1 was uninoculated and treated with distilled water. Group 2 was inoculated and treated with 1ml/kg distilled water daily. Groups 3 (which were inoculated) received daily dose of 10mg/kg body weight of combisunate. Five different concentrations of 100mg/kg, 300mg/kg, 500mg/kg, 800mg/kg and 1000mg/kg body weight of *Moringaoleifera* and *Annonamuricata* leaves extracts were administered to groups 4-8 and 9-13 respectively for five days. All administration was by oral route. Each rat to be sacrificed was withdrawn on the fifth day from the cage and their weight taken five hours to the sacrificial time. The animals were then anaesthetized using a wet cotton wool deepens in chloroform and placed in a desiccator. The thoracic region was opened to expose the heart and fresh blood were quickly taken through cardiac puncture and were collected in a well labelled Heparinized sample bottles and were immediately used for some biochemical assays. Biochemical (Plasma levels of ALT, AST, ALP T.Bil, Creatinin and the concentration of Urea) parameters were conducted at the end of the research to estimate the effects of the extracts on the animal.

2.4 Estimation of Percentage Parasitemia

Thin and thick blood films were prepared with blood collected from the tail of each mouse on the fourth day of inoculation. The thin films were fixed with methanol, stained with Giemsa stain and the percentage parasitemia was determined by microscopic examination using the formula:

$$\%\ Parasitemia = No\ of\ parasitized\ RBC \times 100/No\ of\ total\ RBC$$

The average percentage parasite inhibition was obtained using the formula:

$$Av.\%\ Inhibition = Av.parasitemia\ in\ untreated\ group - Av.parasitemia\ in\ treated\ group\ x\ 100 / Av.parasitemia\ in\ untreated\ group$$

Figure 1: Thick and thin smear slide for qualitative and quantitative estimation of parasitemia

2.5 Data Analysis

Data were entered and analyzed using SPSS software version 20. Descriptive statistics were done. Values are taken as mean ±standard error mean (SEM). A 95% confidence interval was used and P-values <0.05 were considered as statistically significant.

3. Result

3.1 Effect of *Moringa Oleifera* and *Annona Muricata* Leaves Extracts on Parasitemia Level (Suppressive Test)

The results of the standard five days suppressive test of *Moringaoleifera*and*Annonamuricata*leavesextracts on parasitemia levels against P. berghei infected rats were summarized in Table 4.1a-b. The *Moringaoleifera*leaves extract shown little or poor suppressive effects at all levels of dose administration and the effect is does independent.*Annonamuricata*leaves *extracts* presented dose dependent antimalarial effect against P. berghei infection in rats and caused a significant ($p < 0.05$) inhibition when compared to the untreated group. The highest inhibition (95.60%) was observed in leaf with the dose of 1000 mg/kg. However, the rats were not completely cured from the infection in all treatment doses but the treatment doses did significantly ($p < 0.05$) prolong the mean survival time at all dose levels.

Table 4.1 (a): Percentage suppression of Moringaoleiferaagaint P. berghei

S/N	Group	Treatments	Av % parasitemia	Av % Inhibition
1	Normal Control	Food and water only (no inoculation)	0.00±0.00	100.00
2	Negative Control	Inoculated but not treated	23.19±2.49	0.00
3	Positive Control	Inoculated + combisunate 10mg/kg	0.36±0.03	98.48
4	GA1	100mg/kg	14.45±1.01	37.69
5	GA2	300mg/kg	16.34±0.43	29.54
6	GA3	500mg/kg	20.93±1.73	9.75
7	GA4	800mg/kg	16.46±0.65	29.02
8	GA5	1000mg/kg	0.00±0.00	0.00

Where: GA1-GA5 represent 100, 300, 500,800 and 1000mg/kg of Moringaoleifera leaf extracts respectively.

Table 4.1(b): Percentage suppression of Annonamuricataagaint P. berghei

S/N	*GROUP*	*Treatments*	*Av % parasitemia*	*Av % Inhibition*
1	Normal Control	Food and water only (no inoculation)	0.00±0.00	100.00
2	Negative Control	Inoculated but not treated	23.19±2.49	0.00
3	Positive Control	Inoculated + combisunate 10mg/kg	0.36±0.03	98.48
4	GB1	100mg/kg	11.57±0.68	50.11
5	GB2	300mg/kg	3.14±0.27	86.46
6	GB3	500mg/kg	2.05±0.13	91.16
7	GB4	800mg/kg	1.6±0.20	93.10
8	GB5	1000mg/kg	0.70±0.08	96.98

Where: GA1-GA5 represent 100, 300, 500,800 and 1000mg/kg of Annonamuricata leaf extracts respectively.

3.2 Effect of Moringa Oleifera and Annona Muricata leaves Extracts on Some Biochemical Parameters of Albino Rats

The ethanolic leaves extracts in table 4. 2a. Showed a significant increase in the enzymes ALT, AST and ALP in plasma of groups treated with varied concentrations of *Moringaoleifera* as well as in the untreated control group(2). High dose treatment with *Moringaoleifera* increases bilirubin which suggests that the extract may contain toxic substances extractable with organic solvents. But a significant depletion in Urea and Creatinin only occurred at high concentrations. There was a mortality rate of 40% in 800mg/kg extract of *Moringaoleifera* and 100% mortality rate in group treated with 1000mg/kg of *Moringaoleifera.*
The ethanolic leaves extracts in table 4. 2b. Showed a significant decrease in the enzymes ALT, AST and ALP in plasma of groups treated with varied concentrations of *Annonamuricata* as compared to the untreated control group(2).

Table 4.2 (a): Effect of Moringa Oleifera and Annona Muricata Leaves Extracts on Some Biochemical Parameters of Albino Rats

S/N	Group	Treatment	AST	ALT	ALP	T.P
1	Normal Control	Food And Water Only	12.25 ± 0.85^{bc}	12.75 ± 0.75^{bc}	110.50 ± 2.10^{bc}	61.25 ± 1.97^{bc}
2	Negative Control	Untreated	31.75 ± 2.52^{ac}	36.00 ± 2.27^{ac}	299.00 ± 17.89^{ac}	68.50 ± 0.64^{ac}
3	Positive Control	Combisunate	10.50 ± 0.64^{ab}	9.75 ± 1.25^{ab}	111.25 ± 1.31^{ab}	73.75 ± 1.75^{ab}
4	GA1	100mg/kg	19.00 ± 1.08^{abc}	24.75 ± 1.25^{abc}	154.50 ± 4.33^{ab}	74.50 ± 0.86^{bc}
5	GA2	300mg/kg	19.00 ± 0.40^{abc}	22.75 ± 0.47^{ac}	177.00 ± 1.87^{ab}	75.00 ± 1.47^{abc}
6	GA3	500mg/kg	25.00 ± 0.81^{abc}	30.25 ± 0.94^{abc}	212.25 ± 10.11^{bc}	80.00 ± 0.81^{abc}
7	GA4	800mg/kg	31.50 ± 0.64^{ac}	34.25 ± 0.85^{abc}	288.75 ± 2.78^{bc}	84.25 ± 0.47^{abc}
8	GA5	1000mg/kg	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
9	GB1	100mg/kg	17.25 ± 0.85^{ab}	20.25 ± 2.32^{abc}	146.50 ± 3.47^{abc}	77.50 ± 0.50^{abc}
10	GB2	300mg/kg	9.25 ± 0.47^{abc}	10.50 ± 1.32^{ac}	129.00 ± 2.16^{abc}	78.00 ± 0.91^{a}
11	GB3	500mg/kg	13.25 ± 0.94^{bc}	11.00 ± 0.70^{ac}	149.25 ± 1.31^{abc}	80.50 ± 2.62^{abc}
12	GB4	800mg/kg	16.00 ± 0.91^{abc}	15.75 ± 0.85^{ab}	151.50 ± 1.93^{ab}	85.00 ± 1.47^{ac}
13	GB5	100mg/kg	26.75 ± 1.18^{abc}	25.50 ± 1.93^{abc}	189.75 ± 4.75^{abc}	86.25 ± 1.31^{abc}

Data are expressed as Mean ± SEM. n=4. Values found in a column with common superscript letter a, are significantly different (p<0.05) when compared to the normal control. Values with superscript b, are significantly different (p<0.05) relative to the negative control. While values with the superscript c, are significantly different (p<0.05) compared to the positive control.

Table 4.2 (b): Effect of Moringa Oleifera and Annona Muricata Leaves Extracts on Some Biochemical Parameters of Albino Rats

S/N	GROUP	Treatment	ALB	T.BIL	CB	U	CRE
1	Normal Control	Food And Water Only	31.50±1.55^{bc}	10.25±0.62^{bc}	3.75±0.25^{bc}	3.00±0.09^{bc}	51.75±2.32^{bc}
2	Negative Control	Untreated	33.50±1.25 ^{ac}	28.50±2.32 ^{ac}	16.00±1.22 ^{ac}	3.17±0.16 ^{ac}	65.50±2.66 ^{ac}
3	Positive Control	Combisunate	39.75±0.62^{ab}	11.00±0.40^{ab}	5.00±0.40^{ab}	3.00±0.17^{ab}	52.00±9.33^{ab}
4	GA1	100mg/kg	37.25±0.47^{bc}	22.25±0.47^{abc}	13.50±0.28^{abc}	3.30±0.22^{abc}	53.75±1.65^{abc}
5	GA2	300mg/kg	38.75±1.54^{abc}	18.00±0.91^{ab}	11.00±0.57^{bc}	2.42±0.08^{bc}	63.75±3.27^{abc}
6	GA3	500mg/kg	44.50±1.70 ^{ac}	29.00±1.22^{ab}	16.00±0.81^{abc}	2.02±8.32^{abc}	66.50±1.32^{ab}
7	GA4	800mg/kg	48.75±0.47^{abc}	24.00±0.40^{bc}	12.75±0.47^{abc}	3.15±0.06 ^{ac}	57.25±0.85^{ab}
8	GA5	1000mg/kg	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
9	GB1	100mg/kg	41.50±1.04^{abc}	15.25±1.25 ^{ac}	9.25±0.85 ^{ac}	4.27±0.12^{abc}	76.75±3.35^{bc}
10	GB2	300mg/kg	42.75±0.85^{abc}	20.25±0.85^{abc}	13.50±0.64^{abc}	4.10±0.07^{abc}	57.75±5.58 ^{ac}
11	GB3	500mg/kg	49.75±1.79 ^{ac}	10.00±1.47^{abc}	4.50±0.64^{ab}	2.60±0.07^{abc}	50.00±2.12^{abc}
12	GB4	800mg/kg	50.75±1.88 ^{ac}	14.25±0.75^{abc}	8.25±0.75^{abc}	2.27±0.11 ^{ac}	59.75±3.70^{abc}
13	GB5	100mg/kg	50.00±1.77^{ab}	7.50±0.64^{ab}	3.25±0.62^{bc}	3.10±0.16 ^{ac}	46.75±1.70^{abc}

Data are expressed as Mean ± SEM. n=4. Values found in a column with common superscript letter a, are significantly different ($p<0.05$) when compared to the normal control. Values with superscript b, are significantly different ($p<0.05$) relative to the negative control. While values with the superscript c, are significantly different ($p<0.05$) compared to the positive control.

4. Discussion

From the result obtained it has been shown that except for the plant leaves of *Moringa Oleifera*, the plant of *AnnonaMuricata Leaves* ethanolic extracts have anti-plasmodial activities. It has also been shown that *MoringaOleifera* might be toxic to the body of malaria infected rats if the dosage is not regulated. *AnnonaMuricata Leaves* possess Alkaloids which inhibit the growth and functioning of the Plasmodium in the system. Although, more research is needed to investigate the effectiveness and toxicity of this plant, it can be said that this specie can be used in the cure and possible eradication of malaria. Moringaoleifera is commonly addressed as the "miracle tree", and has strong record for curing many diseases, but no concrete write-up has suggest its antimarial properties. This does not mean that there is no probably the presence in the plant of a few molecules which could demonstrate antiplasmodial properties. Dry leaves of the plant do not inhibit beta-hematin in the assay which is often used to screen for antimalarials.

Total Protein measures the amount of two major groups of proteins in the blood: albumin and globulin. A test for total serum protein reports separate values for total protein, albumin, and globulin. An abnormally low total protein result reflects a possible problem with your liver or kidneys indicating that the protein from your diet is not being digested, metabolized and utilized properly by your body. As it's displayed in the table above, the value of Total Protein for the groups showed no significant difference with the normal control except for the groups treated with Moringaoleifera at $p \leq 0.05$ significant levels. An observable trend can also be deduced from the various concentrations of each plant extracts. Similar behavour is observed in Albumin. In general, the values of AST, ALT, ALP, Urea and Creatinin were increased due to inoculation with the P. berghei parasite, but are seen restoring back to normal as it was seen in the non-inoculated group except for groups treated with *Moringaoleifera*. The plant is very rich in proteins, vitamins, polyphenols, minerals, but some recent studies suggest limiting the daily consumption to 70 g because some deterioration in liver functions (increased ALT and AST) has frequently been noticed. Unlike *Annonamuricata* leaves which showed no physiological effects when used as antimalarial drug, *Moringaoleifere* when taken as antimalarial drug can cause rise in some biochemical indices resulting from organ damage and can also aid in promoting the growth of the parasite due to the presence of some proteins and precursors necessary for the enhanced growth of P. berghei parasite in the infected individual.

5. Conclusion

In conclusion, ethanolic extracts of*Annonamuricata*leaves have significant positive effects as antiplasmodial agents against *P. berghei*parasitized rats and have showed no toxicological effect at the varied concentrations so selected for this research. Except for *moringaoleifera* which shows little or no antiplasmodial effects on rats infected with *P. berghei* after five days of treatment with varied concentrations of the ethanolic extracts. Results of groups treated with *moringaoleifera* also show significant rise in biochemical parameters especially, the liver enzyme markers. Hence, consumption of *moringaoleifera* during malaria infection is deleterious as the said "Miracle plant" tends to boast the production of the parasite instead of causing a healing effect.

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