Toxicological and Antiplasmodial Activities of Ethanolic Extracts of Mango (*Mangifera indica*) Leaves and Bitter Cola (*Garcinia kola*) Seeds in Albino Rats

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Abstract: Malaria is one of the most important diseases in the world. The choice for the treatment is highly limited due to drug resistance. Hence, finding the new compounds to treat malaria is urgently needed. The present study aim at investigating the toxicological and anti-plasmodial effects of extracts of *Mangifera indica* and *Garcinia kola* on Albino rat infected with *Plasmodium berghei*. For efficacy test in vivo, standard 5-day suppressive test was carried out. Rats were inoculated with 10/1 ml parasitized erythrocytes of *P. berghei* by intraperitoneal injection. The extracts (100, 300, 500, 800, and 1000 mg/kg) of each plants were given separately to each group and orally once a day for 5 consecutive days. Percentage of inhibition, and biochemical indices were estimated. Combisunate (10 mg/kg) was given to infected rats as reference control while untreated control was given only distilled water. It was found that ethanolic extracts of *Mangifera indica* and *Garcinia kola* at different doses showed dose dependent parasitemia inhibition. Therefore, *Mangifera indica* and *Garcinia kola* exact significant anti-plasmodial activity and prolonged survival time with no toxicity.

Keywords: Toxicological, antiplasmodial, *Mangifera indica*, *Garcinia kola*, *Plasmodium berghei*

1. Introduction

Medicinal plants are those plants that are used (parts and/or extracts, etc.) for the treatment of health challenges. Traditional medicine is the use of medicinal plants instead of pills and capsules. Most time powdered medicines instead of injections are applied on incisions [1]. Practitioners of traditional medication are called the Alternative Medicine Practitioners and/or Herbalists. There are plants that are of medicinal value and the use of these plants for medicinal purpose date back to Verdict period and indeed, the findings of the healing powers of plants is an ancient idea. Citizens of all continents have for long applied poultices and imbibed infusions from extracts of indigenous plants dating back to prehistorically.

The World Health Organization had defined a medicinal plant as any plant which in one way or another of its part can be used for the treatment and prevention of specific diseases of human with no negative effects. It also defined traditional medicine as the sum total of the skills and practices based on the theories, beliefs, and experiences indigenous to different order, whether explainable or not, used in the maintenance of health as well as in the prevention and diagnosis of physical and mental illness [2]. The world health body went further to inform the world that medicinal plants would be the best source of obtaining variety of drugs, therefore such plants should be investigated to understand their properties, safety and efficacy for research with a view for new potential antimicrobial, antifungal and anti-parasitic compounds.

The medicinal value of plants lies in the chemical substances in the parts of the plant, such as seeds, leaves and roots, peels/skin of some fruits. These chemical substances produce definite physiological actions in the human body. Mango, scientifically called *Mangifera indica*, Mongoro in Yoruba, mangolo in in Igbo, and mangoro in Hausa, is a species of flowering plant in the family of Anacardiaceae. Even though the plant is indigenous to the Indian subcontinent, it has varieties approximately about 69 species spread in diversity exists in tropical and subtropical continents of the world, and it was only in the 16th Century this plant was introduce to Africa. The fruits are eaten and used in the production of juice and wine. Application of *Mangifera indica* in traditional medical practice for the treatment of malaria infection had been reported by Tsabang[3]. Hence the extracts of the mango leave is used and have an in vitro activity against plasmodium.

*Garcinia kola* (Heckeler), a member of the Guttiferae family of plant commonly called Bitter cola (English), Orogbo (Yoruba), Nimijigoro among the Hausa. It is a perennial plant that usually grows in the forest area of West and central Africa countries of Cameroon, Ghana, Sierra Leone, including Nigeria, where almost all ethnic groups have a medicinal value of *Garcinia kola*. The nut of the *Garcinia kola* is chewed to release its masticatory bitter content of which is valued for its varied medicinal content, reported in the literature which includes antiplasmodial, antimicrobial, anti-inflammatory, purgative, remedy for guinea-worm infection and for treatment for gastroenteritis, rheumatism, throat infections, bronchitis, and liver disorder [4], [5]. The documented literature contents of phytochemicals obtained from the plant *Garcinia kola* includes Xanthones, coumarine, Biflavonoids, Kolanone, 24-methylene-cyclartenol. [6]. The plant is also used as antidiabetic, antioxidant, chemoprevention of aflotoxin B1 and...
antihapatotoxic activities [7] - [11]. While there are very few literatures on the toxicology and the anti-plasmodial effects of *Mangiferaindica* and *Garcinia kola* in particular, this study will be investigating the toxicology and anti-parasitic activities of these plants at different concentrations in Albino Rats.

1.1 Aim and Objectives

1.1.1 Aim
The Aim of this study is to investigate the toxicological and antiparasomal effects of extracts of Mango (*Mangiferaindica*) leaves and Bitter cola (*Garcinia kola*) seed on Albino rat infected with *Plasmodium berghei*.

1.1.2 Specific Objectives

a) To determine the toxicological effect of extracts of Mango (*Mangiferaindica*) leaves and Bitter cola (*Garcinia kola*) on Albino rat infected with *Plasmodium berghei*.

b) To determine the Antiplasmodial effects of extracts of Mango (*Mangiferaindica*) leaves and Bitter cola (*Garcinia kola*) seeds on Albino rats infected with *Plasmodium berghei*.

c) To determine the effective doses of the above mentioned plant extracts.

1.2 Justification of the Study

Previous researchers had works on antimicrobial activities of extracts of plants and plants products. We intend to delve into the investigation of antiplasmodial activities and toxicological properties of leaf of Mango (*Mangiferaindica*) and seeds of Bitter cola (*Garcinia kola*) on Malaria parasite of animal genus *Plasmodium berghei*. This study will provide the essential information about the medicinal properties and the potency of these plants with respect to antiplasmodial treatment.

2. Materials and Method

2.1 Plant Materials and Sampling Collection and Identification.

The plant species of fresh leaves of *Mangiferaindica*, was obtained from the Botanical Garden of the Department of Plant Science and Biotechnology, University of Port Harcourt. While the fresh seeds of *Garcinia kola* were obtained from the Oil Mill Market in Port Harcourt. The plants parts were all identified by the Departmental Herbarium.

2.2 Preparation of Ethanolic Extracts of Leaves of Mangifera Indica and Seeds of Garcinia Kola

The fresh leaves of *Mangiferaindica* and seeds of *Garcinia kola* were all dried under the sun for two weeks, six hours per day. The dried samples were ground into powdered form using grounding machine. 500g each of the powdered samples were measured into the thimble of a soxhlet extraction apparatus. The solvent used is 100% ethanol and the apparatus was set up to run for hours until the extraction process is complete. The obtained ethanol soluble fractions were transferred into a rotary evaporator and concentrated under decreased pressure at 35°C. The extracts were moved to air-tight vials and maintained at 4°C, away from light.

2.3 Animals and Experimental Conditions

Fifty two Albino rats aged 60 to 100 days and weighing 120 to 140 g were randomly assigned and maintained under standard laboratory conditions, with a 12-h light/dark photoperiod, constant temperature of 23° ± 1° C and relative humidity of 55% ± 10%. The animals were caged in plastic housing units and were acclimatized for a period of 14 days. The experimental animals received water and a balanced diet ad libitum.

2.4 Experimental Design

Before acclimatization, the animals were divided into thirteen groups of four animals each. The Control Group (normal control) received water and a balanced diet ad libitum. The Negative control Group (-ve ctrl) were inoculated but not treated. The positive control Group (+ve ctrl) received 10mg/kg of combisunate after inoculation with 2 x 10^7/ml infected erythrocytes in saline suspensions of 0.5 ml. Combisunate a synergistic antimalarial drug was used as standard antimalarial drug in this study. The drug at 10mg/kg dose was freshly prepared using distilled water and administered orally by gavage. Drug dose, expressed in mg/kg of body weight, was adjusted at the time of administration according to the weight of the rat. Aside the normal control fed with food and water only, the experiments included two control groups. The first control group (-ive ctrl) received only distilled water and the second group (+ve) received 10mg/kg of combisunate^9 per body weight. Five different concentrations of 100mg/kg, 300mg/kg, 500mg/kg, 800mg/kg and 1000mg/kg body weight of *Mangiferaindica* and *Garcinia kola* Ethanolic extracts were administered to groups 4-8 and 9-13 respectively for five days. On the fifth day, blood samples were collected from the caudal vein and thin blood smears were prepared. After Giemsa staining, the air dried prepared slides underwent microscopic examination. The parasitemia detected in the infected control and test animals were recorded at each dose and the percentage suppression of parasitemia was computed based on the obtained values.

2.5 Suppressive Test

The standard 5-day suppressive test against *P. berghei* (ANKA strain) infection in rat was employed [12]. Naïve rats were inoculated by IP injection of 2 x 10^7/ml parasitized erythrocytes. The infected rats were randomly divided into 13 groups of 4 mice per group and treated for 5 consecutive days with 100, 300, 500, 800 and 1000 mg/kg of ethanolic extract of *Mangiferaindica* and *Garcinia kola* accordingly and orally. Two control groups were used: the positive control was treated daily with 10 mg/kg of Combisunate while the untreated group was given Distilled Water. On day 5 of experiment, parasitemia and percentage of inhibition were calculated according to the following formula:
and treated with values with the superscript c, are significantly different (p<0.05) compared to the positive control. When compared to the normal control. Values with superscript b, suppression of 98.19 Mangifera indica suppression of 99.45, the 1000 group. Whereas the suppression for the treated groups relative to the Table 4.1a reveals the average percentage parasitemia Mangifera indica 3.1.1 Table 4.1 reveals the percentage parasitemia (%) parasitemia was calculated using the formula:

\[
\text{% parasitemia} = \frac{\text{number of parasitised red blood cells}}{\text{number of total red blood cells}} \times 100
\]

2.6 Blood Collection

On the fifth day, at the end of administration, each rat was withdrawn from the cage for sacrifice. The rats were anesthetized using chloroform. The thoracic region was opened up to reveal the heart and blood was collected by cardiac puncture. The bloods were collected in well labeled sample bottles (heparinized bottles) and were used for some biochemical assays.

2.7 Data Analysis and Interpretation

The one-way ANOVA was used to analyze and compare the results at a 95% confidence. Values of p < 0.05 were considered significant. Results were expressed as mean ± standard error of mean (SEM).

3. Results

3.1 Evaluation of Anti Malarial Potential

3.1.1 Suppressive Activities of Ethanolic extracts of Mangifera indica and Garcinia kola

Table 4.1a reveals the average percentage parasitemia suppression observed on the fifth day. The results showed a significant difference (p<0.05) in percentage parasitemia suppression for the treated groups relative to the untreated group. Whereas the control group treated with standard drug (combisunate) 10mg/kg dose showed a percentage suppression of 99.45, the 1000mg/kg doses of Mangifera indica and Garcinia kola had percentage suppression of 98.19 and 99.26 respectively.

Table 4.1(a): Percentage Suppression of Mangifera indica and Garcinia kola (control groups).

The combisunate (Negative control) treated group showed an efficacy of 99.45% in stopping the growth of plasmodium Bergheri post treatment.

| Table 4.1a: Percentage Suppression of Mangifera indica and Garcinia kola (control groups). |
|-----------------|-----------------|-----------------|
| S/N | Group | Av % parasitemia | Av % Inhibition |
| 1   | Normal Control | 0.00±0.00 | 100.00 |
| 2   | Negative Control | 18.81±0.79 | 0.00 |
| 3   | Positive Control | 0.10±0.03 | 99.45 |

Key: Normal control = non-inoculated and untreated group; Negative control = inoculated but untreated; Positive control = inoculate and treated with 10mg/kg combisunate

Table 4.1(b): Percentage Suppression of Mangifera indica

The 1000mg/kg dose of Mangifera indica showed inhibition rate close to that of the reference drug (Combisunate). A progressive trend of increase was observed from 100mg/kg (88.89%) to 1000mg/kg (98.19%). Hence it showed dose dependent behavior.

| Table 4.1(b): Percentage Suppression of Mangifera indica |
|-----------------|-----------------|-----------------|
| S/N | Group | Av % parasitemia | Av % Inhibition |
| 4   | GA1 | 2.09±0.12 | 88.89 |
| 5   | GA2 | 2.10±0.19 | 88.84 |
| 6   | GA3 | 1.77±0.11 | 90.59 |
| 7   | GA4 | 0.76±0.09 | 95.96 |
| 8   | GA5 | 0.34±0.03 | 98.19 |

KEY: GA1-GA5 represents the administration of ethanolic extracts doses: 100, 300, 500, 800 and 1000 in mg/kg of Mangifera indica.

Table 4.1(c): Percentage Suppression of Garcinia kola

The extract of Garcinia cola showed also a dose dependent behavior moving from the least 100mg/kg to 1000mg/kg. Comparing its inhibition rate with that of Mangifera indica at high dose of 1000mg/kg indicates that Garcinia (99.26%) might likely possess anti-plasmodia properties more when compared with mangifera indica (98.19%).

| Table 4.1(c): Percentage Suppression of Garcinia kola |
|-----------------|-----------------|-----------------|
| S/N | Group | Av % parasitemia | Av % Inhibition |
| 9   | GB1 | 1.32±0.07 | 92.98 |
| 10  | GB2 | 1.13±0.16 | 93.99 |
| 11  | GB3 | 0.97±0.08 | 94.84 |
| 12  | GB4 | 0.43±0.03 | 97.71 |
| 13  | GB5 | 0.14±0.03 | 99.26 |

KEY: GB1-GB5 represents the administration of ethanolic extracts doses: 100, 300, 500, 800 and 1000 in mg/kg of Garcinia kola.

3.2 Effect of Ethanolic Extracts of Mangifera indica and Garcinia kola on some biochemical Parameters (control groups)

Table 4.2(a): Effect of Ethanolic Extracts of Mangifera indica and Garcinia kola on some biochemical Parameters (control groups).

| Table 4.2(a): Effect of Ethanolic Extracts of Mangifera indica and Garcinia kola on some biochemical Parameters (control groups). |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| S/N | Group | AST | ALT | ALP | T.P | ALB | T.BIL | C.B | U | CRE |
| 1   | Normal Control | 10.50±0.64 | 11.50±0.64 | 98.00±2.16 | 61.50±1.19 | 30.75±1.31 | 8.50±0.64 | 3.25±0.25 | 3.00±0.10 | 55.50±3.17 |
| 2   | Negative Control | 38.25±1.75 | 39.25±0.85 | 221.00±1.82 | 69.00±1.08 | 37.50±0.95 | 28.75±0.85 | 15.75±0.47 | 4.00±0.33 | 68.00±0.91 |
| 3   | Positive Control | 9.75±0.62 | 10.75±0.47 | 127.75±4.36 | 71.50±0.64 | 32.00±0.91 | 10.50±0.28 | 3.50±0.28 | 4.85±0.27 | 80.25±0.85 |

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.

Key: Normal control = non-inoculated and untreated group; Negative control = inoculated but untreated; Positive control = inoculate and treated with 10mg/kg combisunate

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3.2.1 Effect of Ethanolic Extracts of Mangifera indica on some biochemical Parameters
From the result displayed in the table below, it can be deduced that Mangifera indica has hepatoprotective effect as liver enzyme markers- AST, ALT, ALP are seen reducing though it did not show trend of liberality. As the concentration tends toward the highest dose, the leaves extract start showing toxicological effect on the liver notably in AST and ALT parameters. Magifera indica also showed an ameliorating effect on the kidney as values of urea and creatinin are observed to have reduced progressively as the concentration of the extracts increases except for the 1000mg/kg extract of creatinin which showed slight increase.

Table 4.2(b): Effect of Ethanolic Extracts of Mangifera indica on some biochemical Parameters

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<th>ALT</th>
<th>ALP</th>
<th>TP</th>
<th>T.P</th>
<th>CRE</th>
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<th>C.B</th>
<th>U</th>
<th>C.B</th>
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<td>12.00±1.47</td>
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<td>3.00±1.08</td>
<td>18.75±0.85</td>
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<td>GA3</td>
<td>8.00±0.86</td>
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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 (<0.05) relative to the negative control. While values with the superscript c, are significantly different (p<0.05) compared to the positive control.

3.2.2 Effect of Ethanolic Extracts of Garcinia kola on some biochemical Parameters
From the result displayed in the table below, it can be deduced that Garcinia kola also has hepatoprotective effect as liver enzyme markers- AST, ALT, ALP are seen reducing though it did not show trend of liberality. As the concentration tends toward the highest dose, the seed extracts start increasing notably in AST, ALT and ALP parameters. Garcinia kola also showed an ameliorating effect on the kidney as values of urea and creatinin are observed to have reduced progressively as the concentration of the extracts increases. The total bilirubin also showed trend of reduction as the treatment progresses as all levels of administration indicating the healing effect of Garcinia kola.

Table 4.2(c): Effect of Ethanolic Extracts of Garcinia kola on some biochemical Parameters

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<th>T.P</th>
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<td>9.25±0.85</td>
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4. Discussion
The results of this study support the botanical usage of the plants in the treatment of malaria illness. Plants are a major pool of potential antiparasitic and antimicrobial compounds of pharmaceutical needs [13]. Meanwhile, the results partly corroborate claims made in traditional medicine of the antimarial efficacy of these plants. Data in this study indicated that combisunate treatment during the infection almost completely abolished the parasites. Physical signs of illness such as diarrhea and reduced locomotor activity normally seen in malaria-infected rats were absent in combisunate-treated malarial rats and they appeared healthy after five days post treatment. Results with combisunate indicate that the malarial model used in this study is sensitive towards antimalarial agent and therefore justify its use in screening of amimalarial properties from other sources. Among the two plant extracts tested against malaria infection in this study, Garcinia kola exhibited the most pot antimalarial activity. Inhibition on parasitaemia reached almost 92.98% even at the lowest dose of 100mg/kg. However, at the highest dose Garcinia kola exhibited 99.26% (1000mg/kg). The AST level for the normal control and negative control were 10.50±0.64 and 38.25±1.75 respectively. The result revealed that comparing the AST level of the normal control to the negative control, there was a statistically significant increase (p<0.05). Relative to the negative control, the treated groups showed a statistically significant (p<0.05) decrease in the levels of AST. The levels of AST for the treated groups were found decreasing (though with no specific linearity) with increase in doses of plant extracts administered. The ALT levels showed similar behaviour just as the AST. For the treated groups the levels of ALT were significantly (p<0.05) lowered compared to the negative control. The levels of ALT in treated groups were slightly similar (p<0.05) to the level in the normal control. The levels of ALP in the normal control and treated groups vary significantly (p<0.05). The Total protein concentrations were shown to be 61.50±1.19 and 69.00±1.08 for the normal control and treated group respectively. Comparing the T.P concentration for the normal control to the non-treated group showed a statistically significant (p<0.05) increase. The T.P concentrations for the treated groups show no linearity with the normal control as well as the untreated group. The concentration of total Bilirubin for the treated groups showed a progressive decrease with increase in the doses of each category of plant extracts administered. The Urea concentration for the treated groups were significantly (p<0.05) lowered when compared to the untreated group.
The treated groups also responded to dose administration evident by the progressive trend of decrease observed in each plant extracts. The Creatinin concentration for the treated groups were significantly (p<0.05) lowered when compared to the non-treated group except for the positive control that gave a value 80.25±0.85 which is found to be higher than the untreated. The physiological state of the rats treated with Mangifera indica and Garcinia kola were seen returning back to normal from the altered state it went after inoculation with the parasite.

5. Conclusions

Based on these findings, it is clear to us that the oral administration of ethanolic extracts of Mangifera indica and Garcinia kola of dosage range (100–1000 mg/kg) to rats for 5 days significantly suppressed parasitemia of P. berghei in experimental rats with nontoxicity. The implication of this finding is that the ethanolic extracts of Mangifera indica and Garcinia kola possess suppressive antimalarial effects and may therefore serve as potential sources of safe, effective, and affordable antimalarial drugs. They are generally nontoxic and have cardiac, hepato and renal protective abilities when consume within tolerable physiological range.

6. Contribution to Knowledge

The outcome of the study is expected to contribute to the knowledge of pharmacology. The availability of natural products like medicinal plants will greatly help to solve the healthcare problems of rural communities.

- The plant extracts showed a moderate antimalarial property.
- Looking at the dose administered and its effect on the enzyme markers, it can be deduced that Mangifera indica and Garcinia kola has ameliorating effects based on the dose range administered.

7. Recommendations

On the basis of the findings of this research, the following are recommended:
1) Further work should be done to ascertain the use of the plant in preventive malarial therapy.
2) The plants should be used in combination with other plants with known antimalarial activity to understand how this synergy can boost the antimalarial property of these plants.
3) Attempts should be made to isolate the active substance responsible for specific therapeutic action.

References


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