Effects of Aqueous Bark Extracts of *Khaya grandifoliola* and *Enantia chlorantha* on Some Biochemical Parameters in Swiss Mice

Ismaila Olanrewaju Nurain*1, Clement Olatunbosun Bewaji 2

Received: 31.04.2017                                                                                                        Accepted: 13.06.2017

ABSTRACT

**Background:** In this study, the potential side effects of *Khaya grandifoliola* (KG) and *Enatia chlorantha* (EC) were investigated on liver function and hematological parameters of Swiss albino mice infected with malaria.

**Method:** This study was carried out in part in the Department of Biochemistry, Kwara State University, Malete, and in part in the Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria, 2016. Aqueous extracts of both KG and EC were screened for the presence of some phytochemicals using gas chromatography-mass spectrometry. Five groups of eight animals each were used. Group A was administered with only distilled water. Group B was administered with 50 mg/kg body weight of artemisinin-based combination therapy (ACT). Groups C, D, and E were treated with 400 mg/kg body weight of KG, EC and KG-EC combination, respectively. After 28 d, the animals were sacrificed for biochemical analysis.

**Results:** The levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin activities were not significantly different \((P<0.05)\) in all the extract treated animal groups as compared to ACT. However, there was increase in the concentrations of ATL and total bilirubin when compared with that of controls. There was no significant difference \((P<0.05)\) among Hb, RBC, PCV, WBC, lymphocytes, and platelets compared with ACT. However, they increased as compared to the control groups.

**Conclusion:** The aqueous bark extracts of KG and EC either in single or in combined form resulted in hepatotoxicity compared to controls. They also have deleterious effects on hematological parameters of the Swiss mice following administration.

**Keywords:** *Enantia Chlorantha*, Hematologic Tests, *Khaya Grandifoliola*, Liver Function Tests, Meliaceae.

INTRODUCTION

Malaria is still one of the most common infections in developing countries. The parasite that infects humans is *Plasmodium falciparum* and more than 40% of the world population is at risk of clinical incidence. The death of about 400 million people has been reported due to malaria. The disease is especially fatal in children and more than 90% of deaths have been recorded in children in sub-Saharan Africa [1]. The main causes of their death were due to hemolysis that resulted in anemia, effects on cerebrum and complications due to acidosis.

Resistance of these parasites to the several available drugs is one of main issues in controlling the disease. Due to the resistance of *P. falciparum*, chloroquine and sulphadoxine/pyrimethamine are no longer used as therapeutic agents for treatment in Nigeria. This conclusion was reached during a nationwide data monitoring on drug efficacy in Nigeria [2]. Even most malaria vaccines, which would have been the best solution to drug resistance, are still under clinical trials. Due to this resistance by the parasite, there is urgent need for new effective and safe therapeutic agent for the treatment of malaria. It is especially important to develop antimalarial drugs composed of two or more compounds to utilize their synergistic effects. Drug combinations would exert their chemically different modes of actions on the parasite and thereby combat its proliferation. Adoption of herbal medicine as alternative to
synthetic drugs for the treatment of malaria is gaining ground in most parts of the world [3]. However, evaluation of their mechanism of action and side effects is crucial.

Khaya grandifoliola (KG) and Enantia chlorantha (EC) are two common antimalarial agents used in ethnomedicines. In Africa, KG and EC are potent antimalarial plants. Their efficacy as antimalarial has been reported [4], but studies pertaining their side effects and safety are scarce. Blood is a medium of circulation for all the components of the intercellular and intracellular fluids. The major role of blood is to maintain cellular homeostasis [5]. Some biochemical parameters used in assessing the toxicological effects of different substances and therapeutic agents include but are not limited to red blood cells (RBCs), mean corpuscular hemoglobin concentration and white blood cells (WBCs) [6]. Changes in these parameters are good toxicological measures [7]. In the same line, liver is the central processing unit where most of the biochemical activities of the body take place. Drugs and other foreign substances are metabolized in the liver, and it is the main organ used in determining the toxicity of metabolites in the body [8].

Plants and their products have always been considered as possible alternatives to synthetic drugs and are rich sources of new therapeutic agents. Almost all the therapeutic agents for management of various diseases have been either derived from plants or were synthesized based on structural analogs to plant derivatives [9]. Because of the high cost and unavailability of synthetic drugs in poorer regions, many affected individuals or communities embark on adoption of plant remedies [10]. In ethnomedicines, some plants are used for the treatment of a variety of ailments. For instance, while Maytenus senegalensis is used for the management of rheumatism, snake bites and malaria in Africa, it is as well used in South America as anti-inflammatory and analgesic agent. This suggests that a plant could be used for many possible diseases depending on man’s understanding of their components. In addition, this is one of the reasons that therapeutic agents from plants could be suitable choices for the management of the malaria due to their diverse actions [11].

K. grandifoliola, a species of Meliaceae family, is found in various parts of Africa; including Nigeria, Cameroon, Ivory Coast, North and South Africa and many other parts of the world like China and India. Apart from being used as an antimalarial drug, it is also used for management of a cough, convulsion, rheumatism, dermatomycosis, abortion, and stomach ache [12]. E. chlorantha, on the other hand, is commonly found in the forests and coastal areas of West Africa, and the Democratic Republic of Congo. It has potential antiviral and antimalarial properties, previously established in experimental animal models [13]. The problems associated with resistance to many antimalarial drugs and the urgency for the development of new drugs and especially combination therapies are the key reasons for finding new, safe and effective drugs.

In this study, the potential side effects of KG and EC were investigated on liver function and hematological parameters of Swiss albino mice.

MATERIALS AND METHODS

This research work was carried out in part in the Department of Biochemistry, Kwara State University, Malete, and in part in the Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria, 2016.

Reagents and Chemicals

Artemisinin-based Combination Therapy (ACT) was purchased from Ilorin, Nigeria. All the chemicals were of analytical grade.

Plant Materials

The stem bark of both KG and EC were purchased from Ilorin, Kwara State, Nigeria. The plants were identified at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, where the voucher numbers UILH/002/1013 and UILH/003/1066 for KG and EC were deposited, respectively.

Preparation of Extracts

Dried stem bark of KG and EC were pulverized in an electric blender and 400 g of the resulting powder of each plant were extracted in distilled water (4 L) for 48 h. The resulting solution of the plant extract was filtered. The filtrate was concentrated in an oven at a temperature lower than 60 °C.

Gas Chromatography–Mass Spectrometry

The plant powder was extracted in distilled water for 48 h. The resulting mixture was filtered and dried in an oven as described under
“Preparation of Extracts” section. One gram of this powder was further extracted with hexane and filtered. The filtrate was diluted serially in part per million (PPT). The resulting diluted mixture of the extract (100 µL) was pipetted into a vial bottle and inserted into the GC-MS machine. The machine was calibrated and configured as is briefly described next. It employed electron impact ionization (ionizing potential 70eV) and a capillary column (SupelcoSLB-5ms, 30 mx 0.25 mmx 0.25 um film thickness). The ion source temperature was set to 200 °C. The inert gas helium (UHP grade, from Cryogenic Gases) was used as carrier gas, with a linear velocity of 35.9 cm/sec. The injector's temperature was 200 °C and a splitless injection was done. The oven temperature was held at 60 °C for 3 min, then it was heated to 325 °C at 40 °C/min and then was held at 325 °C for 10 min. The transfer line interface temperature was 250 °C. The mass spectrometer was scanned from m/z35 to m/z 400 every 0.5 sec, with a solvent cut time of 3.0 min.

The data was processed with Shimadzu's GCMS Solution software V4.3. Compounds identified were compared with the compounds in the National Institute Standard and Technology database. For each compound, the retention time, name, molecular weight, molecular formula, and percent peak area were determined.

**Experimental Animals**

Forty Swiss mice of both sexes, weighing 14 ± 4 gr, were obtained from the Animal Holding Unit of the Faculty of Veterinary Medicine, The University of Ibadan, and Oyo State, Nigeria. The animals were acclimatized in standard experimental animal cages and were fed with pellets.

All animal treatments and care was based on the recommendations of WHO [14]. The study was approved by the Institutional Ethical Committee of Kwara State University on the use of animals.

**Animal Grouping**

The animals were randomly divided into five groups of eight animals each. The groups were labeled A through group E. They were treated as follows:

Group A: Administered with only distilled water (Control)
Group B: Treated with 50 mg/kg body weight of aqueous extract of KG stem bark
Group C: Treated with 400 mg/kg body weight of aqueous extract of KG stem bark
Group D: Treated with 400 mg/kg body weight of aqueous extract of EC stem bark
Group E: Treated with 400 mg/kg body weight of a combination of aqueous extracts of KG-EC (50%-50%).

All the experimental animals had free access to dry pellets and fresh water *ad libitum* throughout the treatment. The extracts and ACT were administrated orally to the animals every twelve hours for 28 d. After the 28th day of treatment, all animals were sacrificed through jugular puncture under mild anesthesia and the blood was collected in phlebotomy bottles for hematological parameters analysis. The liver of each mouse was removed and placed in plain bottles containing 0.25 M sucrose solution and were stored in a freezer for further biochemical analysis.

**Determination of Hematological Parameters**

Hemoglobin concentration (Hb), packed cell volume (PCV), white blood cell (WBC), platelet count and other hematological parameters were determined by injecting 50 ml of blood samples into an automated blood analyzer. The principle of an automatic multi-parameter blood cell counter machine involves the employment of the differences in characteristics possessed by each of the blood components to distinguish them and estimate their numbers.

**Determination of Liver Enzymes**

Alkaline phosphatase (ALP) was determined by the colorimetric method as was previously described [15]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in the serum were measured by method [16]. The serum concentration of bilirubin was determined as previously described [17].

**Statistical Analysis**

The numerical values were expressed as mean ± S.E.M. The results were analyzed using analysis of variance (ANOVA) and a P<0.05 was considered statistically significant.

**RESULTS**

The results of the phytochemical screening of KG and EC are shown in Table 1 and 2. Thirty compounds were identified in each of these plants. Retention time, compound name, molecular
weight and formula and percentage peak area were recorded for each component. The chemical compound with highest percentage in KG was Urs-12-ene (7.48%) while the one with the lowest concentration was 1-Methoxy-5-trimethylsilyloxyhexane (2.09%) (Table 1). As shown in Table 2, 5, 6-bis (2, 2-dimethylpropylidene)-(Z,Z)-decane (18.85%) was the most abundant substance in EC while 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl) (2H-Cycloprop[a]benzofuran) (0.20%) was the smallest in quantity.

Table 1. Gas chromatography–mass spectrometry (GC-MS) analysis of phytochemicals from Khaya grandi foliola aqueous bark extracts.

<table>
<thead>
<tr>
<th>Peaks</th>
<th>Retention Time</th>
<th>Compound Name</th>
<th>Molecular Weight</th>
<th>Molecular Formula</th>
<th>Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.642</td>
<td>Glycerin (1,2,3-Propanetriol)</td>
<td>92</td>
<td>C₅H₁₀O₃</td>
<td>3.75</td>
</tr>
<tr>
<td>2</td>
<td>5.642</td>
<td>1-tetradecene</td>
<td>90</td>
<td>C₄H₉O₃</td>
<td>2.26</td>
</tr>
<tr>
<td>3</td>
<td>9.250</td>
<td>Benzene propanoic acid (3,5-bis(1,1-dimethyl)-4-hydroxy, methyl ester)</td>
<td>292</td>
<td>C₁₆H₂₆O₃</td>
<td>2.14</td>
</tr>
<tr>
<td>4</td>
<td>9.800</td>
<td>beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl-</td>
<td>342</td>
<td>C₁₃H₂₂O₇</td>
<td>3.25</td>
</tr>
<tr>
<td>5</td>
<td>10.117</td>
<td>Hexanoic acid, propyl ester</td>
<td>158</td>
<td>C₆H₁₄O₂</td>
<td>4.77</td>
</tr>
<tr>
<td>6</td>
<td>10.242</td>
<td>Dimethyl(3-phenylprop-2-enyl)oxyisobutoxy-silane</td>
<td>264</td>
<td>C₁₆H₂₆O₂Si</td>
<td>2.61</td>
</tr>
</tbody>
</table>
The results of the hematological and hepatic parameters are shown in Table 3 and 4. Although there were differences in hemoglobin levels in ACT treated animals as compared to the controls, there was no difference in animals of the extracts treated groups (Table 3). In addition, RBC counts in all treated animals were not significantly different from one another. This same trend was observed on white blood cells, platelets, PCV, neutrophils, and monocytes. There were significant differences in lymphocyte counts for all the treated animals.

The results of liver function enzymes are shown in Table 4. The concentration of ALT, ALP, AST, and bilirubin were not significantly different in the extract treated animal groups as indicated by the superscript numbers in the table. This means that there was no significant difference in the effects of ACT and extracts compared to the ACT treated group.
DISCUSSION

Due to endemic nature of malaria in Africa and many other parts of the world, numerous therapeutic agents have been proposed for its treatment. These agents include both traditional herbal and conventional medicine. Now, in Africa and especially Nigeria, herbal medicine is gaining the attention of medical practitioners due to their effectiveness, safety, and availability. Several ethnomedicinal plants are being used for the management of malaria in different parts of the world [1]. However, despite the efficacy of these plants, investigations concerning their safety are rare. As depicted in Table 1 and 2, some important chemicals are found in both KG and EC.

Due to the abundance of Urs-12-ene compared to other compounds in KG, it may be responsible for the antimalarial potential of this plant. Of course, the importance of bioactive compounds in KG has been previously reported [18]. The abundance of a component does not determine its contribution to the therapeutic properties of a plant. Therefore, any or all of the identified components may be responsible for the antimalarial effects of these plants. Generally, medicinal plants possess numerous substances utilized in the development of new drugs and they may even be precursor from which these drugs are developed [19]. In addition, these medicinal plants have been utilized in most cultures for the management of different ailments [20]. Phytochemicals (chemicals from plants) could be important sources of biologically active agents used for the treatment of many diseases such as malaria, tuberculosis, cancer, diabetes and used as various antioxidants [21].

KG and EC are two very important plants used in the treatment of malaria in Africa. Different blood parameters investigated in this study have been considered useful indices for the evaluation plant toxicity in animals. Administration of any chemical compound for long duration may result in significant change in the structure, function, metabolic transformation and concentration of biomedical enzymes and metabolic pathways. These alterations may occur rapidly or slowly and can lead to different biochemical alterations, producing pathological states [22]. Assessment of hematological parameters is used not only to determine the extent of deleterious effect of formulations on animals but also to explain the efficacy of the plant extracts [23]. Analysis of blood parameters is relevant in risk evaluation as changes in the hematological system have higher predictive value for human toxicity when the data are translated from animal studies [24].

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Hb (g/dl)</th>
<th>RBC (x10^6/mm³)</th>
<th>Neutrophils (%)</th>
<th>Platelets (x1000)</th>
<th>Lymphocytes (%)</th>
<th>PCV</th>
<th>WBC (x1000)</th>
<th>Monocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.37±0.35</td>
<td>8.87±0.47</td>
<td>20.50±0.51</td>
<td>198±1.98</td>
<td>60.67±0.88</td>
<td>40.67±3.33</td>
<td>7.03±0.55</td>
<td>2.00±0.12</td>
</tr>
<tr>
<td>ACT</td>
<td>10.60±0.44</td>
<td>7.38±0.15</td>
<td>19.23±0.48</td>
<td>261±11.27</td>
<td>88.00±2.65</td>
<td>45.33±2.40</td>
<td>13.46±1.11</td>
<td>3.2±0.23</td>
</tr>
<tr>
<td>KG</td>
<td>12.93±0.43</td>
<td>11.43±0.54</td>
<td>21.17±0.75</td>
<td>265±22.60</td>
<td>98.00±1.15</td>
<td>50.00±1.15</td>
<td>12.63±3.01</td>
<td>3.33±0.18</td>
</tr>
<tr>
<td>EC</td>
<td>10.30±0.32</td>
<td>12.03±0.60</td>
<td>21.73±0.23</td>
<td>251±22.87</td>
<td>86.67±1.76</td>
<td>60.67±1.45</td>
<td>14.77±0.21</td>
<td>3.50±0.38</td>
</tr>
<tr>
<td>KG-EC</td>
<td>13.53±0.45</td>
<td>11.67±0.48</td>
<td>21.30±0.98</td>
<td>307±11.41</td>
<td>77.67±2.03</td>
<td>41.33±1.76</td>
<td>12.10±0.98</td>
<td>3.30±0.36</td>
</tr>
</tbody>
</table>

The data were presented as mean±SEM. Values with different superscripts (a-e) are significantly different (P<0.05).

(KG=Khaya grandifoliola, EC= Enantia chlorantha)

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>AST (IU/L)</th>
<th>Total Bilirubin (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>26.33±4.25</td>
<td>48.66±1.76</td>
<td>123.33±7.51</td>
<td>0.46±0.08</td>
</tr>
<tr>
<td>ACT (100%)</td>
<td>163.00±7.50</td>
<td>39.33±1.76</td>
<td>75.66±6.74</td>
<td>1.03±0.08</td>
</tr>
<tr>
<td>KG (100%)</td>
<td>91.66±2.60</td>
<td>45.00±4.16</td>
<td>42.66±4.16</td>
<td>1.60±0.30</td>
</tr>
<tr>
<td>EC (100%)</td>
<td>113.00±4.04</td>
<td>44.33±3.75</td>
<td>61.66±2.90</td>
<td>1.50±0.15</td>
</tr>
<tr>
<td>KG-EC (50%-50%)</td>
<td>133.33±12.34</td>
<td>30.00±3.46</td>
<td>54.66±4.80</td>
<td>1.03±0.08</td>
</tr>
</tbody>
</table>

The data were presented as mean±SEM. Values with superscripts (a-c) are significantly different (P<0.05).

(KG=Khaya grandifoliola, EC= Enantia chlorantha)
The results of the hematological parameters (Table 3), showed that hemoglobin (Hb), red blood cell (RBC), and packed cell volume (PCV) in EC treated group demonstrated no significant difference as compared to ACT treated group. This either is an indication of the safety of the plant extracts singly or in combination, reported already [25]. This is in contrast with a study that plant was moderately toxic as was evident by hematological parameters [26]. Hemoglobin is an iron-containing molecule, which transports oxygen in red blood cells in all vertebrates [27]. Hemoglobin comprises 96% of total content of red blood cells [28] and has high capacity for binding to oxygen [29]. It reacts with oxygen carried in the blood to form oxyhemoglobin during respiration [30]. The effects on erythrocyte indices in this study were in accordance with the findings of a previous paper about the result of administration of aqueous leaf extract of P. nigrescens onto rats [31].

In humans, normal white blood cell count is usually between 4000 and 11000 per L. They make up approximately 1% of total blood volume in healthy adults [32]. White blood cells have the vital role of protecting the body against infections. They are highly resistant to pathogens and enhance adaptability to local environmental and hostile conditions [33]. Low WBC counts can be caused by problems in bone marrow production. Reports on WBC counts have pointed out that increased WBC count is helpful in boosting immune system [26].

The extracts enhanced lymphocyte counts (Table 3) which might refer to their boosting effect on the immune system at the investigated doses. The results of variation in monocytes after treatment demonstrated increases in monocytes in most groups. However, 50/50% KG/EC group showed a significant decrease ($P<0.05$) in monocytes count, which might possibly suggest that combined aqueous extract reduces immunity. Packed cell volume (PCV) also known as hematocrit or erythrocyte volume fraction (EVF) is the percentage of red blood cells in blood. PCV was an indicator of oxygen transport capacity of the blood [33]. Increased packed cell volume points to better oxygen transportation. PCV of all animals in the treated groups increased compared to the controls. Hepatotoxic potentials of the plants and a safety measured must be taken in administering the plants for the management of malaria. In the same line, there was increase in the concentrations of platelets in all treated animal groups compared to the control. Platelets also called thrombocytes are blood cells whose function in addition to the coagulation factors is to stop bleeding [34]. Due to their lack of nucleus, platelets are fragments of cytoplasmic membranes [35]. The main function of platelets is to contribute to hemostasis.

As important as blood, liver is the main site of metabolism in the body. Enzymes are necessary for normal cellular functions including that of the liver [36]. ALT, AST, and ALP are considered indicators of liver functional ability [37]. Compared to ACT treated group, there was no significant difference ($P<0.05$) in the activities of ALT, AST, ALP and bilirubin in the blood of extract treated groups. However, there was significant increase in their concentrations compared to control group. The plant extracts singly or in combination had toxic effect on the hepatic cells. The administration of combine aqueous extracts of KG and EC (50%-50%) also affects these enzymes activities, which is in agreement with previously reported investigations [38].

Aspartate aminotransferase is one of the most important enzymes of liver and in several tissues particularly the heart. AST catalyses the transfer of the amino group of aspartate to α-ketoglutarate. There was no significant difference among the levels in all the treated groups compared with ACT treated cases. ALT catalyses the transfer of the amino group of alanine arriving from muscle to α-ketoglutarate resulting in the production of pyruvate and glutamate. Although the aminotransferases are in the liver and muscle, in pathologic conditions, these enzymes may leak into the blood where they are useful clinical indicators of liver damage. Bilirubin serves as a product from blood catabolism that poses important biological and diagnostic values [39]. Hepatocytes convert bilirubin to a polar molecule by adding glucuronic acid or sulphate molecules to it in a process referred to as conjugation [39]. The levels of ALT, ALP, AST and bilirubin in the extracts treated groups, were not significantly different from ACT treated group.

Thus, the strength of this work is that it shed light on the toxicity of these two plants on hepatic and hematological parameters of mice, which could be extrapolated to human. The limitation,
however, is that the findings did not pinpoint the exact phytochemical that performed the action of the plants.

**CONCLUSION**

In comparison to artemisinin-based Combination therapy, the aqueous bark extracts of KG and EC either in single or in combined form has no significant difference in their effects on the enzymes and hematological parameters. However, these plants possess deleterious effects on both hepatic and hematological parameters of the Swiss mice following their administration. Further studies to establish their effectiveness, optimal dose and safety in humans are required.

**ACKNOWLEDGEMENTS**

The authors are very grateful to Dr. Abdulazeez Abubakar of Medical Laboratory Science, Kwara State University Malete, for his contribution. The authors declare that there is no conflict of interest.

**REFERENCES**


13. Agbaje E, Onabanjo A. Analgesic and antipyretic actions of *Enanta chlorantha* extract in some laboratory animals. NJNPM 1998;2(1):24-5.


24. Olson S, Wang MG, Carafoli E, Strehler EE, McBride OW. Localization of two genes encoding plasma membrane Ca2+-transporting ATPases to


