HEPATO-RENAL EFFECT OF CONCURRENT ADMINISTRATION OF QUININE AND KHAYA SENEGALENSIS IN RATS

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ABSTRACT

Severe malaria is a serious health challenge in tropical developing countries. With the dearth of health facilities in these countries, traditional medicine offers an alternative. The use of herbal remedies alongside orthodox medication, increases the propensity for drug-herb interactions. This can result to potentiation of antiparasitic action and adverse effects. Quinine is an antimalarial that is still used in severe malaria, while the stem bark of Khaya senegalensis is also used for treatment of malaria. Many believe that herbal remedies enhance the effects of orthodox drugs and thus use them concomitantly. This study hypothesized that the concomitant use of quinine and Khaya senegalensis stem bark extract may result in hepato-renal consequences. Male Wistar rats were treated with a combination of quinine and Khaya senegalensis, while quinine and Khaya senegalensis extract were individually administered to two other groups. A normal saline group was also maintained as control. After a five-day treatment, haematological, renal (serum urea, creatinine and electrolytes) and hepatic (ALT, AST and ALP) biomarkers were evaluated. Animals were also weighed daily throughout the treatment. The study showed that there were no statistically significant differences (p>0.05) in renal and hepatic biomarkers. Body weight and haematological indices (RBC, WBC, Platelet, lymphocytes and neutrophils) were also not significantly altered. ALT values ranged between 62-70 mmol/l, while AST ranged between 54-64 mmol/l. Serum urea ranged from 4.2-4.8 mmol/l while creatinine and electrolytes were within limits of the control group. This study shows that the combination of quinine and Khaya senegalensis may not be harmful particularly in short term administration.

Keywords: Drug-Herb Interaction, Quinine, Khaya senegalensis, Hepatic Biomarkers, Renal Biomarkers

INTRODUCTION

Herbal medicines are estimated to be used by 75%-80% of global population, although largely in developing countries due to cultural acceptance and the attached notion of their safety (Kumar et al., 2011). The widespread use of traditional medicine in many low and middle income countries especially in Africa has been linked to inadequate access to orthodox medications (Abdullahi, 2011). Inadequate access to modern medical facilities results in overstretching of available facilities in rural areas, resulting to high disease burden. Malaria is one of such diseases that have stretched medical facilities in the African continent, with a heavy annual death toll, and has also defied all eradication measures. In Nigeria the endemic nature of malaria is widespread through the entire country (Odugbemi et al., 2007). Consequently, patients often resort to the use of herbal medicines for cure, particularly in rural areas due to deeply enshrined beliefs in traditional medicine. Khaya senegalensis (Astraceae) is a popular plant used for its antimalaria properties (Asase et al., 2005; Adebayo and Krettli, 2011). In northern Nigeria, it is known as Madachi and is the African mahogany tree which has several
medicinal and commercial uses. The stem bark of the plant is always severely mutilated owing to well documented therapeutic applications.

In West Africa, herbal drugs are often taken in combination, prior to, or after orthodox antimalaria therapy (Ijarotimi et al., 2010). This is however most often without the knowledge of health personnel, and can result in herb-herb or herb-drug interactions (Falodun, 2010). Most drugs, either herbal or orthodox are administered orally and may thus predispose to pharmacodynamic or pharmacokinetic interactions. Risks particularly associated with pharmacokinetic herb-drug interactions can result in therapeutic failure or drug toxicity (Fasinu et al., 2012).

In many places, quinine is still utilized as an important drug in the treatment of severe malaria. With data failing to show reduced mortality or morbidity following quinine in comparison with artemisinin (PrayGod et al., 2008; Patel et al., 2013), the use of quinine remains a therapeutic option in severe malaria although meta analysis is in favour of artesunate superiority (Sinclair et al., 2012). In sub Saharan Africa, quinine is still the mainstay of therapy for severe malaria (Lubell et al., 2011) and is also sometimes administered in combination with artesunate (Richter et al., 2009; Bartoloni et al., 2010). Thus, given the background of large scale herbal patronage, and the associated mortality and morbidity from falciparum malaria, patients and their relatives are more likely to use proven herbal remedies alongside their prescribed medications with the objective of obtaining additive or synergistic effects to improve therapeutic outcome. The current study consequently investigated the possible effects of the co administration of quinine and Khaya senegalensis on hepatic and renal biomarkers, body weight and hematological parameters in Wistar rats.

MATERIALS AND METHODS

Animals
Young male Wistar rats weighing an average of 149 g obtained from the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used for this study. They were kept in the experimental room for two weeks prior to the experiment to enable them acclimatize. Animal care was according to the NIH and institutional guidelines. The rats were placed on standard feed and water ad libitum for the duration of the experiment.

Drugs and Plant material
Quinine dihydrochloride (Yangshou Xier Kantal, Batch, 120161) was used for the study and dilutions were carried out using distilled water, and this was freshly prepared daily. The stem bark of Khaya senegalensis was collected in the month of June 2012 in Zaria Nigeria and authenticated in the Department of Biological Sciences, Ahmadu Bello University, Zaria by Umar Gallah where a voucher number 900181 was issued from previous herbarium specimen records. Extraction was done using 70% ethanol in water with maceration procedure over 48 hours. The resulting extract was concentrated over a water bath at about 40°C.

Experimental Design
Animals were divided into four groups of six rats each. Group 1 (control) received normal saline (10 ml/kg) while groups 2, 3 and 4 received quinine (40 mg/kg), Khaya senegalensis (50 mg/kg) and quinine (40 mg/kg) + Khaya senegalensis (50 mg/kg), respectively. Doses used in the studies were selected based on previous antimalarial activity in rodent malaria models. Drugs were administered at about 10:00 am daily by oral gavage for five consecutive days. Body weight was measured daily before drug administration.
At the end of treatment, the animals were subjected to an overnight fast. The rats were euthanized with chloroform anaesthesia (Lee, 1989), and blood obtained from the rats were divided into heparinized and plain vacutainers. These were used for the determination of hematological parameters and serum biomarkers respectively. Serum from centrifuged blood was used for the determination of serum urea and creatinine, serum sodium, chloride and potassium, alanine aminotransaminase, alkaline phosphate and aspartate transaminase levels. Markers of hepatic function were determined using standard Reckon Diagnostic kit and an automated Bayer analyzer both using spectrophotometric methods. Electrolytes namely sodium, potassium, chloride as well as serum urea and creatinine were determined as index for renal function. Hematological indices were determined using blood collected in heparinized vacutainers using the automatic hemato analyzer (Sysmex Automated Hematology Analyzer KX-21N).

**Data analysis**

Data obtained from the study was analyzed using one way or repeated measures ANOVA with p<0.05 considered statistically significant. Results are presented as Mean ± SEM in tabular form.

**RESULTS**

The weights of animals that received the individual drugs and the combination of both drugs did not differ significantly throughout the treatment (Table 1). Hepatic enzyme levels were determined as markers of possible alterations in hepatic function. In comparison with the controls, none of the treatment groups showed any significant variation in liver enzyme levels at the end of the experimental protocol (Table 2). The administration of *Khaya senegalensis* in combination with quinine also had no significant effect on serum urea, creatinine and electrolytes. Neither quinine nor the extract independently produced significant changes in renal function at the dose levels used (Table 3). Results from the hematological investigations showed no statistically significant changes when treated groups were compared with the controls. However, decrease in white blood cell and platelet count in groups that received *Khaya senegalensis* either alone or in combination with quinine were observed but these changes were not statistically significant (Table 4).

**Table 1: Effect of five-day oral quinine and *Khaya senegalensis* co-administration on body weight in rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>135.20±10.14</td>
<td>133.80±10.40</td>
<td>138.00±9.95</td>
<td>135.80±9.85</td>
<td>139.40±11.48</td>
</tr>
<tr>
<td>Q 40</td>
<td>135.83±13.03</td>
<td>134.66±12.53</td>
<td>138.16±12.40</td>
<td>135.66±12.72</td>
<td>135.33±13.08</td>
</tr>
<tr>
<td>KS 50</td>
<td>162.80±17.79</td>
<td>154.00±15.40</td>
<td>143.60±12.55</td>
<td>157.80±15.36</td>
<td>158.66±17.59</td>
</tr>
<tr>
<td>QN40+KS50</td>
<td>162.83±16.11</td>
<td>157.66±16.28</td>
<td>155.33±17.04</td>
<td>149.00±17.00</td>
<td>147.33±18.26</td>
</tr>
</tbody>
</table>

Q40=quinine 40 mg/kg; KS 50= *Khaya senegalensis* 50 mg/kg. Data are presented as Mean ± SEM of 6 observations. No significant differences noted, following Repeated measures ANOVA.
Table 2: Effect of five-day oral quinine and *Khaya senegalensis* co-administration on biomarkers of hepatic function in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (IU/l)</th>
<th>AST (IU/l)</th>
<th>ALP (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>67.40±5.12</td>
<td>59.80±4.60</td>
<td>75.80±10.05</td>
</tr>
<tr>
<td>Q 40</td>
<td>70.83±3.63</td>
<td>64.83±7.49</td>
<td>77.50±6.58</td>
</tr>
<tr>
<td>KS 50</td>
<td>69.60±1.36</td>
<td>62.60±2.34</td>
<td>74.80±2.58</td>
</tr>
<tr>
<td>QN40 + KS50</td>
<td>62.00±4.34</td>
<td>54.40±3.03</td>
<td>76.00±4.06</td>
</tr>
</tbody>
</table>

Q40 = quinine 40 mg/kg; KS50 = *Khaya senegalensis* 50 mg/kg. ALT = alanine aminotransaminase, AST = aspartate transaminase, ALP = alkaline phosphatase. Data are presented as Mean ± SEM of 6 observations. No significant differences noted, following One Way ANOVA.

Table 3: Effect of five-day oral quinine and *Khaya senegalensis* co-administration on biomarkers of renal function in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea (mmol/l)</th>
<th>Creatinine (mmol/l)</th>
<th>Sodium (mEq/l)</th>
<th>Potassium (mEq/l)</th>
<th>Chloride (mEq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>4.87±0.31</td>
<td>64.50±6.76</td>
<td>140.20±1.59</td>
<td>3.96±0.16</td>
<td>103.20±2.39</td>
</tr>
<tr>
<td>Q 40</td>
<td>5.08±0.48</td>
<td>73.16±6.51</td>
<td>138.16±0.83</td>
<td>4.26±0.12</td>
<td>100.16±0.74</td>
</tr>
<tr>
<td>KS 50</td>
<td>4.30±0.32</td>
<td>51.40±2.76</td>
<td>136.60±0.67</td>
<td>4.32±0.13</td>
<td>88.00±10.03</td>
</tr>
<tr>
<td>QN40 + KS50</td>
<td>4.28±0.12</td>
<td>66.80±9.55</td>
<td>139.00±1.54</td>
<td>4.16±0.21</td>
<td>101.20±2.37</td>
</tr>
</tbody>
</table>

Q40 = quinine 40 mg/kg; KS50 = *Khaya senegalensis* 50 mg/kg. Data are presented as Mean ± SEM of 6 observations. No significant differences noted, following One Way ANOVA.

Table 4: Effect of five-day oral quinine and *Khaya senegalensis* co-administration on some hematological indices in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>RBC (x10^6)</th>
<th>WBC (x10^3)</th>
<th>PLT (x10^4)</th>
<th>HCT (%)</th>
<th>LYM (%)</th>
<th>NEU (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>6.91±1.64</td>
<td>13.82±1.38</td>
<td>67.18±1.46</td>
<td>40.90±0.93</td>
<td>80.6±1.48</td>
<td>19.40±1.48</td>
</tr>
<tr>
<td>Q 40</td>
<td>6.92±1.74</td>
<td>13.85±1.53</td>
<td>69.71±5.11</td>
<td>40.36±0.70</td>
<td>82.5±2.00</td>
<td>17.48±2.00</td>
</tr>
<tr>
<td>KS 50</td>
<td>6.37±3.94</td>
<td>11.14±1.71</td>
<td>54.60±2.11</td>
<td>38.10±2.22</td>
<td>83.04±2.05</td>
<td>16.58±2.23</td>
</tr>
<tr>
<td>QN40 + KS50</td>
<td>7.12±2.96</td>
<td>11.81±1.67</td>
<td>71.18±4.47</td>
<td>41.68±1.21</td>
<td>80.30±2.09</td>
<td>19.78±2.09</td>
</tr>
</tbody>
</table>

Q40 = quinine 40 mg/kg, KS 50 = *Khaya senegalensis* 50 mg/kg. RBC = red blood cell, WBC = white blood cell, PLT = platelet, HCT = hematocrit, LYM = lymphocyte, NEU = neutrophil. Data are presented as Mean ± SEM of 6 observations. No significant differences noted, following One Way ANOVA.
DISCUSSION

All through the experimental period, no significant changes in body weight were observed across the groups. This is of importance, as changes in body weight can affect energy expenditure and have been reported to be related to catecholamine and thyroid hormone levels (Rosenbaum et al., 2000). The significance of this will be apparent when patients who are overweight and/or have insulin resistance take these therapeutic agents in combination. An earlier study on *Khaya senegalensis* had shown that 50 mg/kg of the aqueous extract did not cause significant changes in serum liver enzymes (Kolawole et al., 2011), which is in agreement with the present study. However, 60 mg/kg of the aqueous extract of *Khaya senegalensis* was found to increase ALT and AST in *Trypanosoma brucei* infection (Ibrahim et al., 2008). Chronic administration of the extract (10-40 mg/kg) also resulted in similar changes in hepatic biomarkers (Abubakar et al., 2009). Thus, the effect elicited by the extract may be dependent on the disease conditions and its mode/schedule of administration.

Quinine as a therapeutic agent is not consistently associated with hepatic damage at antimalarial doses. However, data from clinical experience shows that liver damage could occur particularly when large doses for leg cramps are used (Howard et al., 2003). The absence of deleterious hepatic consequences following the concurrent administration of the extract and quinine, may suggest possible safety with their combination in doses that may exhibit synergy in parasite clearance. The liver being the major organ for biotransformation and involved in other important metabolic functions may thus be unaffected by this drug-herb co-administration.

Hematological parameters were largely unaffected by the extract in this study. Kolawole et al. (2011) did not observe any significant changes in hematological indices with the use of *Khaya senegalensis*. Quinine is known to cause thrombocytopenia which is a severe but rare condition resulting from quinine use (Lefkowitz and Shapiro, 1986). In the present study, the combination of quinine and the extract resulted in apparent but non-significant reduction in platelet count. This observation is thus of importance as clinically utilized doses may result in untoward effects which may be deleterious, particularly with underlying hematological disorders or predispositions. Thrombocytopenia with quinine has however been shown to occur more frequently following intravenous than oral administration of quinine (Tariq et al., 2011). Thus there is reduced likelihood of thrombocytopenia occurring with the oral combination of the quinine and extract.

No changes were observed in the biomarkers of renal function in the groups that received the extract and quinine either singly or in combination. Quinine has been shown to normalize electrolyte imbalances that are associated with falciparum malaria (Etim et al., 2011). Renal function was reported unchanged following 21 day oral administration of aqueous extract of *Khaya senegalensis* with no significant differences between creatinine and urea levels in treated and control groups. El Badwi et al., (2012) also showed that aqueous extract of *Khaya senegalensis* (at
250 and 500 mg/kg) produced no nephrotoxic effects. Since concurrent administration of both quinine and the extract did not significantly alter serum urea, creatinine and electrolytes, renal toxicity may also be considered unlikely.

CONCLUSION

The concurrent oral administration of the ethanolic stem bark extract of *Khaya senegalensis* and quinine did not significantly alter the body weight of animals and did not exhibit hepato-renal or hematological effects. This points to the possible safety of this herb-drug use. The effect of the combination in the disease state of malaria will however require further studies to determine the safety profile with various levels of parasitemia.

REFERENCES


