Toxicity of Antidiarrheal and Spasmolytic Herbal Mixtures Used in Neonates and Infants less than Six Months of Age in Young Rodents

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ABSTRACT
Background: Leaves of Alstonia boonei De Wild. (Apocynaceae), Aristolochia ringens Vahl. (Aristlochiaceae) [Anti-diarrhoea], Allium ascalonicum L. (Amaryllidaceae) and Calliandra haematocephala Haask. (Fabaceae) [Spasmolytic] are components of anti-diarrhoea and spasmolytic herbal mixtures commonly used to treat diarrhoea and abdominal colics in neonates and infants less than 6 months of age in Lagos, Nigeria.

Objective: This study was done to evaluate the acute and sub-acute toxicity of the anti-diarrhoea (aqueous leaf extracts of A. boonei and A. ringens) and spasmolytic (aqueous leaf extracts of A. ascalonicum and C. haematocephala) herbal mixtures in young experimental rodents.

Materials and Methods: Each herbal mixture contained dried leaves of the two plants in equal amounts and was extracted as used traditionally by herbal practitioners. The acute toxicity test was done using young albino mice of both sexes (average weight 15 g) and the 28 day sub-acute toxicity test was done using young albino rats (average weight 120 g) of both sexes to evaluate the safety of the herbal mixtures.

Results: No mortality occurred during the oral acute toxicity study for both the anti-diarrhoea and spasmolytic herbal mixtures. The intraperitoneal acute toxicity study showed mortality at higher doses for both anti-diarrhoea (LD_{50}: 1318 mg/kg) and spasmolytic (LD_{50}: 1175 mg/kg) herbal mixtures. In the 28 day sub-acute toxicity study, both the anti-diarrhoea and spasmolytic herbal mixtures produced no significant increase in the weight of rats, dose-dependent elevations of total cholesterol and low density lipoprotein, significant dose-dependent reductions in platelet counts (p<0.05). The histopathological analysis done after 28 days showed that unlike the spasmolytic herbal mixture, the anti-diarrhoea herbal mixture had dose-dependent fluid congestions of the liver, kidney and brain, and tubular necrosis in the kidneys.

Conclusion: The results of this study show that when administered through the intraperitoneal route in the acute toxicity study, mortality occurred at the higher doses for both the anti-diarrhoea and spasmolytic herbal mixtures. After 28 days, the anti-diarrhoea herbal mixture containing Alstonia boonei and Aristolochia ringens was toxic when administered to young rodents in high doses. The results of this study show evidence of toxicity and these are important safety signals. Therefore, parents, healthcare providers and regulatory authorities should be aware of the potential toxicity of these herbal mixtures, especially when used in the young child without scientific dose finding studies. Pharmacovigilance is essential to detect and document safety of herbal medicines.

Keywords: Neonates, infants less than six months, herbal medicines, toxicity, young experimental rodents.

INTRODUCTION
There has been an increase in the use of naturally occurring traditional medicinal plants which have been culturally accepted, readily available and perceived to be safe (1). Data on the scientific evaluation of the safety and efficacy of medicinal herbs are available but very few of these plants have been studied in the paediatric population (2–4). In the postnatal period, the young infant is susceptible to the toxic effects of drugs because the metabolizing and excretory mechanisms for elimination of drugs are still developing (5). Young children receive herbal medicines which have not been subjected to any scientific evaluation; therefore, studies such as this in young experimental animals are essential to evaluate the safety of herbal medicines, especially in the paediatric population.

In a previous study, we identified herbal medicines prescribed by traditional medicine practitioners and administered to neonates and infants less than six months of age by their mothers in Lagos, Nigeria. Abdominal colics and diarrhoea were the commonest ailments for which herbal medicines were prescribed in this age group. A mixture of Alstonia boonei De Wild. (Apocynaceae) and Aristolochia ringens Vahl. (Aristolochiaceae) was commonly used as anti-diarrhoea agent, while a mixture of Allium ascalonicum L.
(Amaryllidaceae) and *Calliandra haematocephala* Haask. (Fabaceae) was used as spasmyloytic agent by mothers and traditional medicine healers (6).

In folklore medicine, *A. boonei* has traditional therapeutic usefulness and the aqueous ethanolic extract of the stem bark if used at high doses for prolonged periods could be potentially nephrotoxic (7–11). *A. ringens* contains alkaloids, aristolochic acids and has been shown to have antitrypanosomal actions (12). *A. ascalonicum*, whose bulbs contain furostanol saponins, is commonly used to cure earache, fever and pulmonary tuberculosis (13). It is also useful in other microbial diseases, is an antitode for snake venom and an aphrodisiac (14). *C. haematocephala* leaves contains pipecolic acid derivatives and has been documented to have analgesic, antipyretic, anticonvulsant, antiulcer, antioxidant and antimicrobial properties (15).

In this study using young experimental rodents, the objective was to evaluate the acute and sub-acute toxicity of aqueous leaf extracts of anti-diarrhoea herbal mixture containing *A. boonei* and *A. ringens* and spasmyloytic herbal mixture containing *A. ascalonicum* and *C. haematocephala* which are commonly used in neonates and infants less than 6 months in Lagos, Nigeria.

**MATERIALS AND METHODS**

**Plant Material Collection**

The fresh leaves of *A. boonei, A. ringens, A. ascalonicum* and *C. haematocephala* were purchased from the Mushin market, Lagos, Nigeria. The plants were identified and authenticated by Mr. A. Oyebanji of the Herbarium, Department of Botany, University of Lagos, Lagos, Nigeria. Voucher specimens were deposited in the institutional herbarium for reference (Table 1).

**Extraction Process**

The fresh leaves of *A. boonei, A. ringens, A. ascalonicum* and *C. haematocephala* were rinsed with water and air-dried at room temperature separately for 7 days. For the anti-diarrhoeal herbal mixture, dried leaves of *A. boonei* and *A. ringens*, in equal amounts as used traditionally by herbal practitioners, were blended and soaked in distilled water (100 g of each in 4 L) for 24 h. The extract was filtered using a Buchner funnel and Whatman No. 1 filter paper into a beaker and oven-dried at a temperature of 40°C. For the spasmyloytic herbal mixture, *A. ascalonicum* and *C. haematocephala* were treated similarly as the anti-diarrhoeal herbal mixture. The dried extracts were weighed and reconstituted in distilled water (pH = 6.8) to yield a stock concentration of 200 mg/ml.

**Laboratory Animals**

Albino mice (average weight 15 g) and young (4 weeks old) albino rats (average weight 120 g) of both sexes were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria. The animals were housed in clean plastic cages with sub-groups of five animals kept in each cage under standard environmental conditions. Each group was assigned to a specific treatment (control or test). The animals were observed for mortality, morbidity, and behavioral changes daily throughout the study period.

**Table 1: Botanical, Family and Local Names, and Pictures of Plants Used for the Study**

<table>
<thead>
<tr>
<th>Botanical Name</th>
<th>Family</th>
<th>Local Name</th>
<th>Voucher Specimens Reference Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alstonia boonei</em> De Wild.</td>
<td>Apocynaceae</td>
<td>“Ahun” (Yoruba)</td>
<td>LUH6227</td>
</tr>
<tr>
<td><em>Aristolochia ringens</em> Vahl.</td>
<td>Aristlochiaceae</td>
<td>“Ako-igun” (Yoruba) and “Dumandutsee” (Hausa)</td>
<td>LUH4061</td>
</tr>
<tr>
<td><em>Allium ascalonicum</em> L.</td>
<td>Amaryllidaceae</td>
<td>Alubosa elewe (Yoruba)</td>
<td>LUH6218</td>
</tr>
<tr>
<td><em>Calliandra haematocephala</em> Haask.</td>
<td>Fabaceae</td>
<td>Tude (Yoruba)</td>
<td>LUH6217</td>
</tr>
</tbody>
</table>
conditions. They were acclimatized to animal house conditions for 7 days before dosing and allowed free access to drinking water and standard rodent pellet diet (Pfizer, Lagos, Nigeria). The laboratory animals were fasted for 14 h overnight before the experiment. All experimental protocols were done according to the Guide for the Care and Use of Laboratory Animals (16, 17).

Acute Toxicity Test

Oral Acute Toxicity

For the anti-diarrhoea and spasmolytic herbal mixtures respectively, mice were randomly divided into six groups of five animals per group. In the experimental groups (5 groups), graded doses of the mixture of aqueous extracts (250, 500, 1000, 2000 and 4000 mg/kg) were administered orally for the animals using oro gastric tube. The sixth group was the control group and received 0.5 ml of distilled water orally. After administration, the mice were observed for mortality, behavioural changes (restlessness, dullness, agitation) and signs of toxicity at 1 h, 2 h, 4 h, 6 h, 24 h and subsequently once daily for 14 days post-treatment (18).

Intraperitoneal Acute Toxicity

For the anti-diarrhoea and spasmolytic herbal mixtures respectively, mice were randomly divided into six groups of five animals per group. Graded doses of the mixture of aqueous extracts (50, 100, 200, 400 and 500 mg/kg) were administered intraperitoneally to the animals in the five experimental groups. The sixth group was the control group and the animals were administered 0.5 ml distilled water intraperitoneally. After administration, the mice were observed for mortality, behavioural changes (restlessness, dullness, agitation) and signs of toxicity at 1 h, 2 h, 4 h, 6 h, 24 h and subsequently once daily for 14 days post-treatment (19).

Sub-Acute Toxicity Test

For the anti-diarrhoea and spasmolytic herbal mixtures respectively, 25 young albino rats of both sexes were randomly divided into five groups of five animals each. Group 1 (control) received 1 ml of distilled water administered orally, while Groups 2 to 5 received graded doses of the aqueous extracts (50, 100, 200 and 400 mg/kg) for 28 days using oro gastric tubes. The animals were inspected daily for the appearance of signs of toxicity and the percentage mortality for each dose was noted for the 28 days study period. The rats were weighed weekly during the observation period. On the 29th day, blood was collected from the animals by ocular puncture using a capillary tube and stored in ethylenediaminetetraacetic acid (EDTA) bottles for haematological analysis and heparinized bottles for blood chemistry analysis (18, 19). For both the acute and sub acute toxicity tests, the median lethal dose (LD$\textsubscript{50}$) was estimated using the log dose-probit analysis method (20). All experimental groups had control groups except the body weight changes where baseline data was used as control data.

Histological Analysis

The animals were sacrificed by cervical dislocation under halothane anaesthesia and the brain, kidney and liver were isolated. The tissue samples for histological examination were passed through the process of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. To ensure good fixation, the tissues were trimmed to about 5 mm thickness. The tissues were fixed in 10% formal saline and were then transferred to 50% alcohol (70, 80, 85, 95 and 100%) for 2 h. Alcohol was removed from the treated tissues by titrating them through first an equal mixture 100% (absolute) alcohol and xylene for 1 h each in that order. Infiltration was carried out twice by passing each tissue through molten paraffin wax in an oven at a temperature of 30 °C for one and a half hours each. The tissues so embedded in molten paraffin wax were later placed on a wooden block and trimmed to size. Serial sections 20 μm thick were made using a rotatory microtome. The cut sections were then floated in a warm water bath at a temperature of 30–40 °C and were placed on slides. Eight sections were obtained from each treated organ from each animal. Four samples were placed on each slide. Microscopic examination was done by using varying magnifications of 10, 40, 100 and 400 to determine if the samples were properly fixed on the slides. Following staining, mounting of sections was carried out using dimethyl paraaffinate xylene (DPX) as a mounting agent, after which microscopic examination was done (Haematoxylin/eosin, 20 μm – 400×) (21, 22).

Statistical Analysis

This was performed using GraphPad Prism 5 statistical package (GraphPad Software, San Diego MA, USA). Data were expressed as means of five replicates and reported as mean±SEM. Data were subjected to two-way ANOVA followed by Bonferroni post hoc test.

RESULTS

Acute Toxicity

In the oral acute toxicity tests, no animal deaths were recorded with the aqueous extracts of the anti-diarrhoea (A. boonei and A. ringens) and spasmolytic (A. ascalonicum and C. haematocephala) herbal mixtures at doses of 250, 500, 1000, 2000 and 4000 mg/kg within 24 h post-administration and the subsequent observation period of 14 days. The behavioural changes observed at the higher doses (2000 and 4000 mg/kg) include mild irregular changes in breathing pattern (anti-diarrhoea herbal mixture) and increased motor activity followed by reduction (spasmolytic herbal mixture).

Acute toxicity study using the intraperitoneal route of administration showed mortality at the higher doses of 1000 and 2000 mg/kg of both anti-diarrhoea (A. boonei and A. ringens) and spasmolytic (A. ascalonicum and C. haematocephala) herbal mixtures. Surviving animals were normal at 24 h. The LD$\textsubscript{50}$ was estimated to be 1318 mg/kg for the anti-diarrhoea herbal mixture and 1175 mg/kg for the spasmolytic herbal mixture. Tables 2–5 show the dose and signs of acute toxicity in mice 24 h after oral and intraperitoneal administration of the anti-diarrhoea and spasmolytic herbal mixtures.
Tables 6 and 7 shows mean body weight changes in sub-acute (oral) toxicity study in albino rats after 28 days of administration of aqueous leaf extracts of anti-diarrhoea herbal mixture containing *A. boonei* and *A. ringens* and spasmolytic herbal mixture containing *A. ascalonicum* and *C. haematocephala*. There were no significant changes in body weights in all dose ranges compared to baseline weights (*p*>0.05). Tables 8, 9, 10 and 11 shows blood chemistry and haematological parameters in sub-acute (oral) toxicity study in albino rats after 28 days of administration of aqueous leaf extracts of anti-diarrhoea herbal mixture containing *A. boonei* and *A. ringens* and spasmolytic herbal mixture containing *A. ascalonicum* and *C. haematocephala*. The 28 days sub-acute toxicity study showed that both herbal extracts possessed a high safety profile. Compared to the control group, there were significant low density lipoprotein (LDL) elevations at 100 mg/kg (*p*<0.05) and 200 mg/kg.

### Table 2: Oral Acute Toxicity Study in Mice after 24 Hours of Administration of Aqueous Leaf Extract of Anti-diarrhoea Herbal Mixture Containing *A. boonei* and *A. ringens*

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Dose (mg/kg)</th>
<th>D/T</th>
<th>Signs of Toxicity Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0/5</td>
<td>NIL</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0/5</td>
<td>NIL</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>0/5</td>
<td>NIL</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>1/5</td>
<td>Mild changes in breathing pattern</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>1/5</td>
<td>Mild changes in breathing pattern</td>
</tr>
<tr>
<td>6</td>
<td>0.5 ml distilled water</td>
<td>0/5</td>
<td>NIL</td>
</tr>
</tbody>
</table>

*D/T: Number of mice deaths/Total number of mice (n=5).

### Table 3: Oral Acute Toxicity Study in Mice after 24 Hours of Administration of Aqueous Leaf Extract of Spasmolytic Herbal Mixture Containing *A. ascalonicum* and *C. haematocephala*

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Dose (mg/kg)</th>
<th>D/T</th>
<th>Signs of Toxicity Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0/5</td>
<td>NIL</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0/5</td>
<td>NIL</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>0/5</td>
<td>NIL</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>0/5</td>
<td>Increased followed by reduced motor activity</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>3/5</td>
<td>Increased followed by reduced motor activity</td>
</tr>
<tr>
<td>6</td>
<td>0.5 ml distilled water</td>
<td>0/5</td>
<td>NIL</td>
</tr>
</tbody>
</table>

*D/T: Number of mice deaths/total number of mice (n = 5).

### Table 4: Intraperitoneal Acute Toxicity Study in Mice after 24 Hours of Administration of Aqueous Leaf Extract of Anti-diarrhoea Herbal Mixture Containing *A. boonei* and *A. ringens*

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Dose (mg/kg)</th>
<th>D/T</th>
<th>Signs of Toxicity Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0/5</td>
<td>NIL</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0/5</td>
<td>NIL</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>0/5</td>
<td>NIL</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>1/5</td>
<td>Mild changes in breathing pattern</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>1/5</td>
<td>Mild changes in breathing pattern</td>
</tr>
<tr>
<td>6</td>
<td>0.5 ml distilled water</td>
<td>0/5</td>
<td>NIL</td>
</tr>
</tbody>
</table>

*D/T: Number of mice deaths/Total number of mice (n=5).

### Table 5: Intraperitoneal Acute Toxicity Study in Mice after 24 Hours of Administration of Aqueous Leaf Extract of Spasmolytic Herbal Mixture Containing *A. ascalonicum* and *C. haematocephala*

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Dose (mg/kg)</th>
<th>D/T</th>
<th>Signs of Toxicity Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0/5</td>
<td>NIL</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0/5</td>
<td>NIL</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>0/5</td>
<td>NIL</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>2/5</td>
<td>Increased motor activity followed by reduced motor activity</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>3/5</td>
<td>Increased motor activity followed by reduced motor activity</td>
</tr>
<tr>
<td>6</td>
<td>0.5 ml distilled water</td>
<td>0/5</td>
<td>NIL</td>
</tr>
</tbody>
</table>

*D/T: Number of mice deaths/total number of mice (n = 5).

### Sub-Acute Toxicity

Tables 6 and 7 shows mean body weight changes in sub-acute (oral) toxicity study in albino rats after 28 days of administration of aqueous leaf extracts of anti-diarrhoea herbal mixture containing *A. boonei* and *A. ringens* and spasmolytic herbal mixture containing *A. ascalonicum* and *C. haematocephala*. There were no significant changes in body weights in all dose ranges compared to baseline weights (*p*>0.05). Tables 8, 9, 10 and 11 shows blood chemistry and haematological parameters in sub-acute (oral) toxicity study in albino rats after 28 days of administration of aqueous leaf extracts of anti-diarrhoea herbal mixture containing *A. boonei* and *A. ringens* and spasmolytic herbal mixture containing *A. ascalonicum* and *C. haematocephala*. The 28 days sub-acute toxicity study showed that both herbal extracts possessed a high safety profile. Compared to the control group, there were significant low density lipoprotein (LDL) elevations at 100 mg/kg (*p*≤0.05) and 200 mg/kg.

### Table 6: Mean Body Weight Changes in Sub-Acute (Oral) Toxicity Study in Albino Rats after 28 Days of Administration of Aqueous Leaf Extract of Anti-diarrhoea Herbal Mixture Containing *A. boonei* and *A. ringens*

<table>
<thead>
<tr>
<th>Doses of Extract of Anti-diarrhoea Herbal Mixture</th>
<th>Mean Body Weight Changes (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Body Weight Changes (g)</td>
<td>50 mg/kg</td>
</tr>
<tr>
<td>Baseline</td>
<td>30.08±1.21</td>
</tr>
<tr>
<td>Week 1</td>
<td>31.86±1.27</td>
</tr>
<tr>
<td>Week 2</td>
<td>35.78±1.28</td>
</tr>
<tr>
<td>Week 3</td>
<td>37.72±1.14</td>
</tr>
<tr>
<td>Week 4</td>
<td>41.46±1.48</td>
</tr>
</tbody>
</table>

*Values are mean±SEM (n=5). *p*>0.05; Baseline data served as control.*
mg/kg ($p<0.0001$). Also, there were significant LDL and total cholesterol elevations at 50 mg/kg compared to the control group ($p<0.05$). The levels were lower at 100 mg/kg and 400 mg/kg compared to the 50 mg/kg group ($p<0.0001$).

Haematological parameters in the group that received anti-diarrhoeal herbal mixture showed significant reductions in platelet counts in the 50 mg/kg, 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.0001$) compared to the 100 mg/kg group. Compared to the control group, with the spasmylocytic herbal mixture, there was a significant drop in platelet count in the 50 mg/kg group ($p<0.05$). There was also significant reductions in platelet counts in the 200 mg/kg ($p<0.05$) and 400 mg/kg groups ($p<0.0001$) compared to the 50 mg/kg group.

**Histopathology**

The histopathological analysis done after the 28 days sub-acute toxicity test revealed that the spasmylocytic herbal mixture had no adverse effect on the brain, kidney and liver. The anti-diarrhoeal herbal mixture showed dose-dependent toxicity. At 50 mg/kg, the mixture caused congestions in the liver and kidney, with mild cerebral oedema in the brain, while at 100 mg/kg only mild cerebral oedema was seen. At 400 mg/kg, acute tubular necrosis was observed in the kidney of the rats (Figures 1–3).
Table 11: Haematological Parameters in Sub-Acute (Oral) Toxicity Study in Albino Rats after 28 days of Administration of Aqueous Leaf Extract of Spasmolytic Herbal Mixture Containing *A. ascalonicum* and *C. haematocephala*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>50mg/kg</th>
<th>100mg/kg</th>
<th>200mg/kg</th>
<th>400mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell count (10³/µL)</td>
<td>14.5±0.05</td>
<td>7.5±0.03</td>
<td>9±0.07</td>
<td>9.5±0.07</td>
<td>4.4±0.06</td>
</tr>
<tr>
<td>Red blood cell count (10³/µL)</td>
<td>4.3±0.06</td>
<td>6.47±0.02</td>
<td>6.53±0.07</td>
<td>6.63±0.03</td>
<td>6.59±0.02</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>8.9±0.08</td>
<td>11.2±0.06</td>
<td>12.1±0.06</td>
<td>11.5±1.02</td>
<td>12±0.09</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>36.5±0.02</td>
<td>41±0.08</td>
<td>40.4±0.12</td>
<td>37.3±0.06</td>
<td>40.8±0.03</td>
</tr>
<tr>
<td>Platelet count (10³/µL)</td>
<td>570±0.45</td>
<td>361±0.49</td>
<td>468±0.52</td>
<td>555±0.32</td>
<td>672±0.41</td>
</tr>
<tr>
<td>Mean cell volume (fl)</td>
<td>85.1±1.05</td>
<td>63.5±0.09</td>
<td>62±0.08</td>
<td>56.4±1.02</td>
<td>62±1.04</td>
</tr>
<tr>
<td>Mean cell haemoglobin (Pg)</td>
<td>20.6±0.12</td>
<td>17.3±0.15</td>
<td>18.5±0.12</td>
<td>17.3±0.04</td>
<td>18.2±0.02</td>
</tr>
<tr>
<td>Mean cell haemoglobin concentration (g/dl)</td>
<td>24.3±0.10</td>
<td>27.3±0.14</td>
<td>29.9±0.16</td>
<td>30.8±0.12</td>
<td>29.4±0.09</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=5). *p<0.05 vs. control group; *p<0.05, *p<0.0001 vs. treatment dose 50 mg/kg; two-way ANOVA followed by Bonferroni post hoc test.

**DISCUSSION**

Traditional medicine plays a strong role in child healthcare in a large proportion of sub-Saharan African populations where patterns of healthcare-seeking behaviour for sick children by caregivers vary from place to place (23, 24). Like in previous studies which investigated the toxicological evaluation and safety of herbal medicines (3,4,25-27), the present study investigated the safety of the aqueous extract of herbal mixtures used by herbal medicine practitioners for treatment of diarrhoea (*Alstonia boonei* and *Aristolochia ringens*) and abdominal colics (*Allium ascalonicum* and *Calliandra haematocephala*) in neonates and infants less than 6 months using young experimental animal models. We used very young experimental animals because the findings are important and could be extrapolated to young humans who take these herbal medicines. Differences in age and species have been shown to affect outcomes in drug therapy (28–30).

The oral acute toxicity study showed minor dose-dependent changes in breathing pattern, increased motor activity followed by a reduced motor activity. In respect of the intraperitoneal route of administration, dose-dependent toxicity with mortality was observed. This suggests involvement of central respiratory and motor nervous system mechanisms (18, 19, 31).

No mortalities were observed in respect of the 28 days sub-acute oral toxicity study. Compared to the baseline weights, there were no significant changes in weight of rats of both sexes treated with the increasing doses of both anti-diarrhoea and spasmyolytic herbal mixtures. Previously, the aqueous extract of *A. boonei* stem bark has been shown to significantly increase the percentage of the body weight gain in male rats (but not in the female) in a dose-dependent manner (32). Female rodents have been shown to be relatively more sensitive to chemicals than males and several studies have shown male specific effects.
Fig. 2: (A) Histological slides of the control brain from animals receiving distilled water orally; (B) Histological slides of the brain (mild cerebral oedema) in sub-acute (oral) toxicity study in albino rats after 28 days of administration of aqueous leaf extract of anti-diarrhoea herbal mixture containing *A. boonei* and *A. ringens* (50 mg/kg; Haematoxylin/eosin, 20 μm, 400×); (C) Histological slides of the brain (mild cerebral oedema) in sub-acute (oral) toxicity study in albino rats after 28 days of administration of aqueous leaf extract of anti-diarrhoea herbal mixture containing *A. boonei* and *A. ringens* (100 mg/kg; Haematoxylin/eosin, 20 μm, 400×).

Fig. 3: (A) Histological slides of the control liver from animals receiving distilled water orally; (B) Histological slides of the liver (congestion) in sub-acute (oral) toxicity study in albino rats after 28 days administration of aqueous leaf extract of anti-diarrhoea herbal mixture containing *A. boonei* and *A. ringens* (50 mg/kg; Haematoxylin/eosin, 20 μm, 400×).

The mechanism for this gender differences are not clear; however, the loss of body weight in females compared to males has been attributed to water loss induced by echitamine and echitamidine, which are chemical constituents of *A. boonei* that have been reported to have diuretic and hypotensive properties (36–38). Contaminations with heavy metals such as lead, mercury, and arsenic or bacteria have also been reported (39–42).

Like in the oral acute toxicity tests and the 28 days sub-acute oral toxicity test in this study, previous studies showed that the aqueous extract of *A. boonei* stem bark (a component of the anti-diarrhoea herbal mixture in this study) did not cause mortality in experimental rats (8). In this present study, we observed significant dose-dependent changes in lipid profile that will need further evaluation. We did not find significant leucocytosis and anaemia, increased total white blood cell counts and decreased haematocrit and *A. boonei* induced elevation of liver enzymes that was found in previous studies. However, we observed significant dose-dependent reductions in platelet counts compared to the control group animals that.
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received both herbal mixtures (43,44). The implications for the haemostasis in the young infant who received these herbal medicines need to be evaluated further.

The anti-diarrhoea extract consisting of A. boonei and A. ringens had some toxic effects on the organs. We observed dose-dependent congestion in the liver, mild cerebral oedema in the brain, and acute tubular necrosis in the kidney of the treated rats. Although the aqueous leaf extracts of the plants was used in this study, the findings on the liver differ from that of some previous studies which reported the hepatoprotective effect of the ethanolic extract of A. boonei stem bark (11, 37, 45–48). Mechanisms of drug-induced hepatotoxicity vary; dose related or directly mediated. Susceptible individuals may also be affected (49). Drug-induced intrahepatic cholestasis is a major factor in hepatic toxicity. Reactive metabolites play major roles in the pathogenesis of herb-induced toxicity (50) and involve covalent binding of reactive oxygen species and other reactive intermediates to macromolecules, resulting in severe harmful hepatotoxic reactions (50–52).

Several case reports in Europe, Asia, and China and Africa indicate increasing incidence of herbal medicine-induced nephrotoxicity. Acute renal failure from the use of herbal remedies is said to account for about 30–35% of all cases of acute kidney failure in Africa and nephrotoxins from herbal medicines have been implicated (53–55). A. ringens has been established to contain alkaloids, flavonoids, tannins, terpenoids, cardiac glycosides, essential oils, aristolochic acid and other phytochemicals that are of biological importance. Some of these constituents are also potent nephrotoxic agents (35, 56). “Chinese herb nephropathy” results from the nephrotoxic properties of aristolochic acid (57,58). The major renal injury is extensive interstitial fibrosis with tubulatrophe and loss (59). Exposure to aristolochic acid increases the risk for urothelial malignancies (60). Recently, it was shown that aristolochic acid tubulotoxicity results in defective activation of antioxidative enzymes, mitochondrial damage and apoptosis; the renin angiotensin system (RAS) usually is not involved (61). Generation of reactive intermediates through metabolic activation of herbal constituents, with resultant reactive intermediates that bind covalently to DNA and proteins, lead to organ toxicity, mutagenicity and carcinogenicity (62). The innate recovery ability of the renal tubular epithelial cells (RTEC) after acute injury has been shown to be severely diminished in aristolochic acid-induced acute tubular necrosis (AA–ATN); this effect may be partly due to reduced epidermal growth factor (EGF) expression (63,64).

Damage to the brain may arise during the use of herbal medicines. The developing central nervous and immune systems in young infants may make them more sensitive to the adverse effects of herbs (31). Cerebral oedema, an excessive accumulation of fluid within the intra cellular or extra cellular spaces of the brain, was observed in this study. It is typified by the occurrence of a large quantity of fluid in the brain; and if neglected, it can be life threatening and can trigger severe brain impairment (65). In previous studies, aqueous extract of A. boonei was found to be toxic in high doses (44). Excitotoxicity, which is the action of neurotransmitters in the propagation of injury within the central nervous system, could be involved as elevated vanilmandelic acid concentration occurs in brain tissue following the administration of ethanolic extract of A. boonei in rats (66).

Evidence about the relation of the age at injury to the central nervous system and subsequent outcome has been a subject of debate. Ability of the young brain to recover is important in predicting outcome and the age of the individual at the time of the insult may play an important role in recovery. Evidence from animal experiments has tended to suggest that brain lesions in young animals are generally better tolerated than those sustained at a later age. On the one hand, the consequences of generalised metabolic disturbance and infection seem to be more severe in the young (67).

CONCLUSION

In summary, we have identified toxic effects with the anti-diarrhoeal herbal mixture containing aqueous leaf extracts of Alstonia boonei and Aristolochia ringens. There are many such herbal mixtures used in the young child without scientific safety assessments. Therefore, health care providers, especially those that are involved in paediatric care, should be aware of evidence regarding potential benefits or harm of herbal medicinal agents when used by neonates and infants. Regulatory authorities should demand pre-marketing tests of safety and effectiveness. Parents should inform clinicians of any herb or dietary supplement that they are giving their children. Pharmacovigilance is essential to document safety of herbal medicines for the development of an effective regulatory framework for safe and effective use of herbal products in the very young child.

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Conflict of Interest

None declared.

REFERENCES


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