Toxicological evaluation of aqueous extracts of Clematis hirsuta and Rhamnus prinoides

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Abstract

*Clematis hirsuta* leaves and *Rhamnus prinoides* roots have a long history of medicinal use in Nyeri County, Kenya. However, there is no evidence to back up their safety. The acute and subacute toxicity of *Clematis hirsuta* aqueous leaf and *Rhamnus prinoides* aqueous root extracts in *Wistar* rats was investigated in this study. Changes in body weight, feed and water consumption were used as toxicity indicators in the acute toxicity study, while changes in weight, biochemical and hematological parameters were used as toxicity indicators in the subacute toxicity study. The data from the acute toxicity study was summarized as mean±standard deviation and analyzed using the unpaired Student’s t-test. The data from the subacute toxicity study was summarized as mean ±standard deviation and analyzed using Two Way ANOVA and Tukey’s post hoc test. The significance level was set at *p*≤0.05. The extracts reduced feed and water consumption in rats but caused no physical signs of toxicity or death, nor did they have any significant effects on weight, biochemical, or hematological parameters when compared to controls. These findings indicate that oral administration of *Clematis hirsuta* aqueous leaf and *Rhamnus prinoides* aqueous root extracts to *Wistar* rats is generally nontoxic.
1. Background

Many developing countries rely on traditional herbal medicine to meet their primary healthcare needs. Traditional herbal medicine has grown so rapidly that discussions about how to incorporate it into mainstream healthcare are ongoing. Traditional medicine is popular among consumers due to its ease of use, low cost, proven efficacy, and perceived fewer side effects. Secondary metabolites, which plants use for defense, protection against pests, diseases, insects, pathogenic organisms, and to attract pollinators, are credited with the pharmacological efficacy of traditional herbal medicine. Nonetheless, the safety of herbal medicine remains a hotly debated topic. Long-term studies, for example, have revealed the toxic properties of the Lamiaceae plant family monoterpenes. Phytotoxic metabolites from the legume *Ascochyta* and *Phoma*, child deaths in the Caribbean, Mexico, and Central America, due to *Thevetia peruviana*, *Chenopodium ambrosioides*, and *Argemone mexicana*, consumption have also been reported. These studies demonstrate that, contrary to popular belief, not all traditional medicines are safe for consumption, emphasizing the importance of toxicological research.

*Clematis hirsuta* is a small climbing shrub that grows to a height of 4 m in Kenya, Uganda, Tanzania, and Saudi Arabia. It is a member of the Ranunculaceae family, with oppositely arranged compound leaves and panicles of white or yellow flowers. In Asia and Africa (Ethiopia), it is used to treat inflammatory conditions and manage pain. Abdel-Kader and colleagues isolated some compounds from the pet ether extract of *C. hirsuta*, including β-amyrin, stigmasterol glycoside, lupeol, (S)-(−)-5-hydroxymethyl-2(5H)-furanone, β-sitosterol, (S)-(−)-dihydro-5-
(hydroxymethyl)-2(3H)-furanone, and oleanolic acid \[21\]. *C. hirsuta*’s ability to inhibit various disease-causing pathogens as well as its free radical scavenging properties have also been reported \[20\][22][23][24].

*Rhamnus prinoides* on the other hand is a dense evergreen shrub that can grow to 7.5 m in height. It is found in India, Ethiopia, Eritrea, South Africa, Cameroon, Congo, Angola, and Kenya and belongs to the Rhamnaceae family \[25\][26]. It has culinary and medicinal applications \[26\][27][28][29][30]. It is used as a bittering agent in the preparation of tella and tej, which are traditional fermented beverages from East Africa \[27\][28]. Medicinally, it is used to treat respiratory tract, skin, and gastrointestinal tract infections \[26\]. Quercetin, emodin, 4-hydroxy, 4-methyl pentanone, anthracene derivatives, geshoodin, and 4-ethyl-benzoate are among the phytochemical compounds identified in the plant \[26\][31][32][33].

Leaves and roots of *Clematis hirsuta* and *Rhamnus prinoides* respectively are used to treat type 2 diabetes in Nyeri County, Kenya \[34\]. However, no consideration has been given to the safety of these medicinal plants. The current study sought to determine the safety of *Clematis hirsuta* leaves and *Rhamnus prinoides* roots in *Wistar* rats by measuring changes in weight, biochemical, and hematological parameters.

2. Methods

2.1. Ethical considerations

The University of Nairobi’s Biosafety, Animal Use and Ethics Committee was consulted for ethical approval \[REF FVM BAUEC/2022/392\].

2.2. Materials

Leaves of *Clematis hirsuta* and roots of *Rhamnus prinoides* were sourced from Nyeri County. The equipment used in this experiment included an electric mill (Tencan, China), analytical balance (Mettler, USA), Muslin cloth (Sciencequip, Kenya), Centrifuge (Sigma Aldrich, USA), freeze drier (Bioevopeak, USA), *Wistar* rats (Animal holding unit, University of Nairobi), polypropylene cages (Industrial area, Nairobi), and Hematology and Biochemistry kits (Mindray, China).

2.3. Medicinal plant collection and identification

Herbalists from Nyeri County assisted in collecting leaves and roots of *C. hirsuta* and *R. prinoides* respectively. Plant specimens were collected and stored in a plant press until they could be identified botanically. After that, the specimens were identified at the University of Nairobi Herbarium, where voucher specimens were also deposited.

2.4. Preparation of medicinal plant extracts

Plant materials were carefully sorted and washed under running tap water. After one week of shade drying, the plant material was pulverized using an electric mill, weighed and mixed with distilled water in a ratio of 1:10. Hot water
extraction was performed to mimic the traditional method of decoction preparation. The mixture was strained using a muslin cloth, filtered, and centrifuged at 3000 rpm for 5 minutes, and the supernatant was collected. The supernatant was freeze-dried (Mondulyo-UK) and stored at -20°C pending toxicological testing.

2.5. Experimental animals

The animal holding unit of the Department of Veterinary Anatomy and Physiology, Faculty of Veterinary Medicine, University of Nairobi provided fifty-six 8-10-week-old female *Wistar* rats. The rats were randomly selected, weighed (180-200 grams), and transferred to polypropylene cages. They were then marked to permit identification and a period of five days was used to acclimatize the rats to the laboratory conditions (20-25°C and a 12-hour light and dark cycle). Water and food (Unga rat pellets) were provided *ad libitum*.

2.6. Acute toxicity

The acute toxicity of *C. hirsuta* aqueous leaf and *R. prinoides* aqueous root extracts in twenty-one female *Wistar* rats was determined using the Limit Test Dose of the Up and Down Procedure Method [35]. Each group contained five rats, and the maximum dose of each extract was 2000 mg/kg. Only distilled water was given to the rats in the control group. Over a two-week period, the control and treatment rats’ feed and water consumption, as well as their mean body weight gain, were measured.

2.7. Subacute toxicity

The sub-acute toxicity of *C. hirsuta* aqueous leaf and *R. prinoides* aqueous root extracts was determined using the Up and Down Procedure on 35 female *Wistar* rats [36]. Each group had five animals, with extract doses of 25 mg/kg, 75 mg/kg, and 225 mg/kg. The control group only received distilled water. The weight, hematological, and biochemical parameters of the control and treatment animals were measured over a four-week period [36].

2.8. Statistical analysis

In the acute toxicity protocol, the student's test was used to compare differences in the mean body weight gain, and the mean feed and water consumption of control and treatment group rats. Differences in hematological and biochemical changes between treatment and control group rats were assessed using two-way ANOVA and Tukey's post hoc test in the sub-acute toxicity protocol. The level of significance was set at *p*≤0.05. GraphPad Prism (9.0) and GenStat (15th edition) were used as statistical tools.

3. Results

3.1. Acute toxicity
3.1.1. Mean feed consumption

Figures 1 A and B compare the mean feed consumption in rats given 2000 mg/kg of *Clematis hirsuta* aqueous leaf extract or *Rhamnus prinoides* aqueous root extract to the mean feed consumption in rats given only distilled water.

![Graph showing mean feed consumption](image)

*Figure 1*. Feed consumption in *C. hirsuta* aqueous leaf extract (A) or *R. prinoides* aqueous root extract (B) treated rats relative to feed consumption in control group rats after 14 days.

The mean feed consumption of rats in the *C. hirsuta* treatment group was significantly lower than that of rats in the control group \((p=0.0222)\). **Figure 1A**. The mean feed consumption of rats in the *R. prinoides* treatment group was significantly lower than that of rats in the control group \((p=0.0014)\). **Figure 1B**.

3.1.2. Mean water consumption

Figures 2 A and B compare the mean water consumption in rats given 2000 mg/kg of *Clematis hirsuta* aqueous leaf or *Rhamnus prinoides* aqueous root extracts to the mean water consumption in rats given distilled water only.
The mean water consumption of rats in the *C. hirsuta* treatment group did not differ significantly from that of rats in the control group (*p*=0.1187). Figure 2A. The mean water consumption in the *R. prinoides* treatment group was significantly lower than that in the control group (*p*=0.0010). Figure 2B.

3.1.3. Mean body weight

Figures 3 A and B compare the mean body weight gain in rats given 2000 mg/kg of *Clematis hirsuta* aqueous leaf or *Rhamnus prinoides* aqueous root extracts to the mean body weight gain in rats given only distilled water.
There was no statistically significant difference in mean body weight gain between C. hirsuta treatment group rats and control rats \((p=0.2181)\). Figure 3A. However, the mean body weight of rats given 2000 mg/kg of R. prinoides aqueous root extract was significantly higher than the mean body weight of rats given only distilled water \((p=0.0351)\). Figure 3B.

3.1.4. Physical examination of rats for signs of toxicity

There were no changes in the colour of the skin, hair, eyes, mucous membranes, respiration or motor activity. No convulsion, tremors, salivation, diarrhoea, lethargy, sleep or mortality was observed during the first 24 hours and after 14 days of treatment with Clematis hirsuta aqueous leaf extract or Rhamnus prinoides aqueous root extract. The results show that the LD\(_{50}\) for both extracts was >2000 mg/kg.

3.2. Subacute toxicity

During the experimental period for the subacute toxicity study (28 days), there were no signs of toxicity or mortality in treated rats. The results showed that the extracts’ LD\(_{50}\) was > 225 mg/kg. Furthermore, the weight gain in extract-treated rats was not significantly different \((p>0.05)\) from the weight gain in untreated (control) rats in the second week of treatment. Figure 4A. Rats given 75 mg/kg and 225 mg/kg C. hirsuta aqueous leaf extract gained significantly more weight than untreated (control) rats in the third week of treatment \((p=0.0011 \text{ and } p=0.0011 \text{ for } 75 \text{ mg/kg and } 225 \text{ mg/kg respectively}). Figure 4A. C. hirsuta aqueous leaf extract-treated rats gained significantly more weight than untreated (control) rats in the fourth week of treatment \((p=0.0003, p<0.0001, \text{ and } p=0.0004 \text{ for } 25\text{mg/kg, 75mg/kg, and 225mg/kg respectively}}). Figure 4A.
respectively). Figure 4A.

Figure 4. Percentage of weight gain in experimental animals after 28 days of different doses of *C. hirsuta* aqueous leaf extract (A) and *R. prinoides* aqueous root extract (B)

Rats given the highest dose of *R. prinoides* aqueous root extract gained significantly more weight than untreated (control) rats in the first and second weeks of treatment. Figure 4B. (Week 1, *p*=0.01, and Week 2, *p*<0.0001). Rats given 75mg/kg and 225 mg/kg of *R. prinoides* aqueous root extract gained significantly more weight than untreated (control) rats in the third week of treatment (*p*<0.0001, and *p*<0.0001 for 75mg/kg and 225 mg/kg respectively). Figure 4B. Rats given 25 mg/kg, 75 mg/kg, and 225 mg/kg doses of *R. prinoides* aqueous root extract gained significantly more weight than untreated (control) rats in the fourth week of treatment. Figure 4B. (*p*=0.0490, *p*<0.0001, and *p*<0.0001 for 25mg/kg, 75mg/kg, and 225mg/kg respectively)

3.3. Hematological values

Table 1 shows the effect of different doses of *C. hirsuta* aqueous leaf and *R. prinoides* aqueous root extracts on hematological parameters in rats after 28 days. The hematological parameters examined in this study included white blood cells, mean corpuscular hemoglobin concentration, eosinophils, neutrophils, mean corpuscular hemoglobin, hematocrit, lymphocytes, monocytes, mean corpuscular volume, basophils, red blood cells, hemoglobin, and platelets.

Table 1. Toxicological effect of *Clematis hirsuta* aqueous leaf and *Rhamnus prinoides* root extract on various hematological parameters in rats after 28 days
### 3.4. Biochemical values

Table 2 shows the effect of different doses of *C. hirsuta* aqueous leaf and *R. prinoides* aqueous root extracts on biochemical parameters in rats after 28 days. Among the biochemical parameters studied were bilirubin, urea, albumin,
total protein, alanine aminotransferase, sodium, alkaline phosphatase, creatinine, aspartate aminotransferase, chloride, and potassium.

Table 2. Toxicological effect of Clematis hirsuta aqueous leaf and Rhamnus prinoides root extract on various biochemical parameters in rats after 28 days.

<table>
<thead>
<tr>
<th>Biochemical values</th>
<th>Control</th>
<th>25 mg/kg</th>
<th>75 mg/kg</th>
<th>225 mg/kg</th>
<th>25 mg/kg</th>
<th>75 mg/kg</th>
<th>225 mg/kg</th>
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<tr>
<td></td>
<td>CH</td>
<td>CH</td>
<td>CH</td>
<td>CH</td>
<td>CH</td>
<td>CH</td>
<td>CH</td>
</tr>
<tr>
<td>Albumin (10^3 µL)</td>
<td>43.91±0.46</td>
<td>41.18±0.93</td>
<td>43.42±1.26</td>
<td>43.02±1.05</td>
<td>41.57±0.46</td>
<td>43.78±0.86</td>
<td>42.27±1.52</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>191.20±5.35</td>
<td>137.60±9.66</td>
<td>140.70±3.31</td>
<td>200.20±10.17</td>
<td>161.50±11.27</td>
<td>186.30±28.26</td>
<td>151.60±4.52</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>180.50±10.85</td>
<td>167.90±11.66</td>
<td>170.69±6.93</td>
<td>166.80±16.95</td>
<td>174.70±10.70</td>
<td>180.30±15.60</td>
<td>177.70±34.95</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>446.10±58.20</td>
<td>428.10±10.74</td>
<td>433.40±13.48</td>
<td>435.70±51.20</td>
<td>442.70±12.81</td>
<td>384.50±10.37</td>
<td>471.80±80.98</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>0.84±0.12</td>
<td>0.92±0.04</td>
<td>0.96±0.12</td>
<td>0.88±0.09</td>
<td>1.00±0.10</td>
<td>0.86±0.13</td>
<td>0.84±0.08</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>113.70±1.64</td>
<td>118.29±2.47</td>
<td>114.50±1.33</td>
<td>115.99±0.63</td>
<td>116.90±0.50</td>
<td>118.30±0.57</td>
<td>114.70±0.13</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>32.00±2.98</td>
<td>24.00±1.00</td>
<td>26.60±1.29</td>
<td>28.00±1.23</td>
<td>31.00±1.92</td>
<td>30.00±0.89</td>
<td>27.00±0.51</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>10.65±0.33</td>
<td>6.56±0.21</td>
<td>7.25±0.10</td>
<td>6.76±0.44</td>
<td>8.07±0.51</td>
<td>10.33±0.98</td>
<td>9.58±0.63</td>
</tr>
<tr>
<td>Na (mEq/L)</td>
<td>137.30±1.59</td>
<td>142.00±0.94</td>
<td>140.60±0.79</td>
<td>142.50±0.46</td>
<td>144.60±0.34</td>
<td>140.80±0.44</td>
<td>144.80±1.21</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>81.30±2.41</td>
<td>72.80±1.48</td>
<td>80.94±1.58</td>
<td>80.18±1.59</td>
<td>76.00±0.32</td>
<td>80.62±0.86</td>
<td>74.28±2.23</td>
</tr>
<tr>
<td>Urea (µmol/L)</td>
<td>12.45±0.85</td>
<td>9.55±0.23</td>
<td>10.73±0.31</td>
<td>11.00±0.45</td>
<td>9.08±0.34</td>
<td>11.28±0.10</td>
<td>9.61±0.31</td>
</tr>
</tbody>
</table>

Values are shown as (mean ± SEM, n = 5). Means where the superscripts are different across the rows are significantly different at p<0.05. mg/kg; milligrams per kilogram, CH; Clematis hirsuta, RP: Rhamnus prinoides, ALT; alanine aminotransferase, AST; aspartate aminotransferase, K; potassium, ALP; alkaline phosphatase, Na; Sodium

Treatment of rats with 25mg/kg, 75 mg/kg, or 225 mg/kg doses of C. hirsuta aqueous leaf extract or 25mg/kg, 75 mg/kg, or 225 mg/kg doses of R. prinoides aqueous root extract was associated with a non-statistically significant change (p>0.05) in the mean albumin, bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) values relative to untreated (control) rats. (Table 2). The mean alkaline phosphatase (ALP) and potassium levels in rats treated with C. hirsuta aqueous leaf extract or R. prinoides aqueous root extract were significantly lower (p<0.05) than in untreated (control) rats (Table 2). The mean creatinine levels in rats treated with R. prinoides aqueous root extract were significantly lower (p<0.05) than in untreated (control) rats (Table 2).

The mean alkaline phosphatase (ALP) and potassium levels in rats treated with C. hirsuta aqueous leaf extract or R. prinoides aqueous root extract were significantly lower (p<0.05) than in untreated (control) rats (Table 2). The mean chloride value was significantly higher (p<0.05) in rats treated with 75 mg/kg R. prinoides aqueous root extract than in untreated (control) rats (Table 2). The mean creatinine levels in rats treated with 25 mg/kg, 75 mg/kg, or 225 mg/kg R.
prinoides aqueous root extract or 25 mg/kg C. hirsuta aqueous leaf extract were significantly lower (p<0.05) than in untreated (control) rats (Table 2).

The sodium levels were significantly higher (p<0.05) in rats given 25 mg/kg and 225 mg/kg C. hirsuta aqueous leaf extract or 25 mg/kg R. prinoides aqueous root extract than in untreated (control) rats (Table 2). The mean total protein values in rats treated with 25 mg/kg, 75 mg/kg, or 225 mg/kg R. prinoides aqueous root extract or C. hirsuta aqueous leaf extract were significantly lower (p<0.05) than in untreated (control) rats (Table 2). The mean urea levels in rats treated with 25 mg/kg of C. hirsuta aqueous leaf extract or 25 mg/kg of R. prinoides aqueous root extract were significantly lower (p<0.05) than in rats that were not treated (control) (Table 2).

4. Discussion

When evaluating the safety of medicinal plants, it is critical to determine the nature, significance, and level of exposure at which untoward effects occur [37][38]. The current study investigated the safety of Clematis hirsuta aqueous leaf and Rhamnus prinoides aqueous root extracts in rats.

Toxicology data on C. hirsuta presented in the current study was a mixed bag. For instance, in the acute toxicity study, rats given 2000 mg/kg of C. hirsuta aqueous leaf extract exhibited weight gain relative to control group rats with no physical signs of toxicity after 24 hours or 14 days. Ironically, C. hirsuta-treated rats had lower feed and water consumption rates than control group rats. For toxic substances, the expectation is that the ability of the rats to feed and consume water will be compromised. If this is the case, it is not expected that the rats will have a higher weight gain than the control group rats as was observed in this study. More studies are required to understand this discrepancy. In the subacute toxicity protocol, there were no significant changes in weight gain, hematological, or biochemical parameters between Clematis hirsuta-treated rats and controls. Because this may be the first study on the toxicology of Clematis hirsuta, it may be important to compare our findings with toxicological data from other medicinal plants within the genus Clematis. The genus Clematis, for example, generally contains protoanemonin, a compound which irritates the skin and mucous membranes [39][40]. A 34-year-old man who used Clematis chinensis for wrist pain developed hypo- and hyperpigmentation, as well as pruritic erythema [41]. According to another report, Clematis chinensis can cause bullae, abdominal cramping, palpitations, inflammation, hypersalivation, bloody diarrhoea, blistering, inflamed eyes, vomiting, and weakness [42]. Two milligrams of Clematine (an alkaloid isolated from Clematis flammula) caused frequent urination, general tremors, altered respiration, palpitations, convulsions, coma, and death in guinea pigs [43]. The poisonous nature of Clematis glycinoides limits its use in veterinary medicine [44]. The aerial parts of Clematis mandshurica and its essential oil had LD₅₀ values of 51.85 mg/kg and 3.28 ml/kg respectively in mice [45]. Convulsions, confusion, and dizziness have been reported following the use of Clematis virginiana [46]. Beggars in ancient Rome applied Clematis spp. juice on their hands to cause blisters in order to gain more sympathy from people [46].

The administration of R. prinoides aqueous root extract to Wistar rats did not result in any physical signs of toxicity during the first 24 hours or after 14 days and was associated with significant weight gain relative to the control. However, the
feed and water consumption in *R. prinoides*-treated rats were lower than in the control group rats. Furthermore, there were no significant differences in weight gain, hematological, or biochemical parameters between *R. prinoides* aqueous root extract-treated rats and controls. There have been few studies on the safety of *R. prinoides*.[47][48][49]. Kamanja found that *R. prinoides* aqueous root, methanol-water, and chloroform extracts were toxic to *Artemia salina*, with LC₅₀ values of 6921.05 g/mL, 214.33 g/mL, and 133.33 g/mL, respectively [47]. In human oral epidermoid cancer cell lines, Koch and colleagues found an IC₅₀ of more than 20 µg/mL for *R. prinoides* root bark chloroform extract [48]. Another study found that *R. prinoides* aqueous leaf extract caused mortality ranging from 11.67% to 88.33% in the mendi termite (*Macrotermes subhyalinus*) when treated for 24 to 72 hours [49].

This study did not evaluate the effects of *C. hirsuta* aqueous leaf and *R. prinoides* aqueous root extracts on major organs in *Wistar* rats. Future work should examine the extracts' pathological effects on major organs in rats, as well as their phytochemical composition and pharmacological properties.

5. Conclusions

The findings reveal that *C. hirsuta* aqueous leaf extracts and *R. prinoides* aqueous root extracts are generally safe when orally administered in *Wistar* rats.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of interest

The author(s) declare that there is no conflict of interest regarding the publication of this paper.

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