Safety of potentised dilutions of Rhus toxicodendron in Wistar albino rats

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Safety of potentised dilutions of Rhus toxicodendron in Wistar albino rats

Abstract
Background: Rhus toxicodendron (Rhus tox.) is a well-known homoeopathic medicine used to treat inflammatory disorders supported by historical and modern scientific evidence. Objective: The safety testing of 6C, 30C and 200C potencies of Rhus tox. through acute and sub-acute oral toxicity tests as per the Organisation for Economic Cooperation and Development test guidelines. Methods: For the acute oral toxicity study, Rhus tox. (2000 μL/kg) was administered to rats and observed for 14 days. For the subacute oral toxicity study, Rhus tox. (200 μL/kg) was administered for 28 days and additional recovery groups were included to monitor reversibility, persistence, or delayed occurrence of toxic effects for 14 days post-treatment. Histopathological assessments of vital organs were done to detect any signs of toxicity. Results: No mortality occurred during the acute toxicity study at a dose of 2000 μL/kg, indicating an oral LD50 of Rhus tox. >2000 μL/kg. In the sub-acute toxicity study, Rhus tox. administration for 28 days showed no adverse clinical signs, with normal weight gain and feed intake in treated animals. No adverse changes were noticed in the biochemical and haematological parameters of Rhus tox. treated rats. Furthermore, no abnormalities were observed in gross and histopathological examinations of vital organs. Conclusion: The study found that Rhus tox. in 6C, 30C and 200C potencies exhibited a safe toxicological profile, supporting its beneficial pharmacological effects.

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Safety of potentised dilutions of *Rhus toxicodendron* in Wistar albino rats

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**Abstract**

**Background:** *Rhus toxicodendron* (*Rhus tox.*) is a well-known homoeopathic medicine used to treat inflammatory disorders supported by historical and modern scientific evidence. **Objective:** The safety testing of 6C, 30C and 200C potencies of *Rhus tox.* through acute and sub-acute oral toxicity tests as per the Organisation for Economic Cooperation and Development test guidelines. **Methods:** For the acute oral toxicity study, *Rhus tox.* (200 µL/kg) was administered to rats and observed for 14 days. For the subacute oral toxicity study, *Rhus tox.* (200 µL/kg) was administered for 28 days and additional recovery groups were included to monitor reversibility, persistence, or delayed occurrence of toxic effects for 14 days post-treatment. Histopathological assessments of vital organs were done to detect any signs of toxicity. **Results:** No mortality occurred during the acute toxicity study at a dose of 2000 µL/kg, indicating an oral LD₅₀ of *Rhus tox.* >2000 µL/kg. In the sub-acute toxicity study, *Rhus tox.* administration for 28 days showed no adverse clinical signs, with normal weight gain and feed intake in treated animals. No adverse changes were noticed in the biochemical and haematological parameters of *Rhus tox.* treated rats. Furthermore, no abnormalities were observed in gross and histopathological examinations of vital organs. **Conclusion:** The study found that *Rhus tox.* in 6C, 30C and 200C potencies exhibited a safe toxicological profile, supporting its beneficial pharmacological effects.

**Keywords:** Acute toxicity, Homoeopathy, Organisation for Economic Cooperation and Development, *Rhus tox.*, Safety, Subacute toxicity

**Introduction**

Homoeopathy, a form of complementary and alternative medicine founded by Samuel Hahnemann (1755–1843), is widely utilised.¹ Homoeopathic remedies, which are highly diluted and prepared in a specific manner, are believed to activate the body’s natural healing process to treat and/or prevent a variety of diseases.² However, the safety of homoeopathic medicines remains in question despite their increasing worldwide market demand.³ Because of the nature and variability of the source material, it is necessary to ensure the safety and quality of homoeopathic medicines at the international, national and regional levels to protect consumers.⁴

*Rhus toxicodendron* (*Rhus tox.*) is a homoeopathic medicine derived from the plant *Rhus toxicodendron* Linn. or *Toxicodendron pubescens*, commonly known as poison ivy.⁵ Homoeopathic dilutions of *Rhus tox.* are employed to treat a range of clinical conditions, particularly those involving inflammation and pain, such as articular and muscular pains and stiffness, skin conditions such as eczema, hives and rashes and flu-like.⁶ *Rhus tox.* in mother tincture and potentised dilution form has been reported to exhibit anti-inflammatory,⁷,⁸ anti-arthritic⁹,¹⁰ and immunomodulatory¹¹ activities. *Rhus toxicodendron* plant is reported to cause type IV hypersensitivity and contact dermatitis in significant numbers of people, which is attributed to an allergen named Urushiol.¹² Therefore, while considering the therapeutic benefits of *Rhus tox.* medicine, the allergenic properties of its plant source material should also be known. However, as of now, there is a lack of in vivo studies reporting the safety of homoeopathic medicine, *Rhus tox.* Nevertheless, some in vitro studies have been reported.¹³

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The present study aims to investigate the acute and sub-acute toxicity of *Rhus tox*. in 6C, 30C and 200C potencies according to Organisation for Economic Cooperation and Development (OECD) test guidelines (with slight modifications) in Wistar albino rats. These potencies were chosen since they are frequently mentioned in scientific literature and are included in the National List of Essential Ayush Medicines, 2022. Given that the majority of homeopathic medicines are used clinically as alcohol-based liquid dilutions, the dose in this study was measured in microliters per kilogram (µL/kg) body weight rather than the milligrams per kilogram (mg/kg) often employed in the OECD.

**Materials and Methods**

**Homeopathic medicines**

6C, 30C and 200C potencies of *Rhus tox*. (Batch No. 0204) and dispensing alcohol – 90% (Batch No. 8345) manufactured by Hahnemann Publishing Co. Pvt. Ltd, Kolkata, as per Homoeopathic Pharmacopoeia of India were used in this experiment.

**Experimental animals**

Healthy male and female Wistar albino rats obtained from the West Bengal livestock development corporation, Kolkata, were employed as per the recommendations of OECD guidelines. Animals were housed in polycarbonate cages (3 per cage) under standard conditions (12-h light-dark cycle, 23 ± 2°C room temperature and 55–65% relative humidity) with access to a standard pellet diet (VRK Industries Pvt. Ltd, Maharashtra, India) and water *ad libitum*. Bedding material, autoclaved corn cob, procured from Sparconn Life Sciences Pvt. Ltd, Karnataka, was used, and feed was obtained from Vishnu traders, Roorkee, Uttarakhand. The institutional animal ethical committee of Dr Anjali Chatterji Regional Research Institute for Homoeopathy, Kolkata, approved the study on 6 March 2021 (proposal no. DACRRIH/CPCSEA/IAEC/2021/006). The use and care of experimental animals were carried out in accordance with the Committee for the Control and Supervision of Experiments on Animals recommendations. The animals had health examinations by the veterinarian before the initiation of the study, and only healthy animals were employed in the experiments. Furthermore, the entire investigation was reported in agreement with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, maintaining animal-based scientific research’s integrity. As this is a safety study, controls (diseased animals) were not applicable.

**Blinding**

Investigators and researchers were blinded during the experiment and a veterinarian was responsible for the blinding procedures. Decoding was done after analysing the results.

**Acute oral toxicity study**

An acute oral toxicity study of *Rhus tox.* was carried out in female rats as per the OECD 423 guideline. 30 rats, (each weighing 150–200 g) were randomly assigned to five groups, with six animals each. Group I (control) was given distilled water; Group III (vehicle control) was given 90% dispensing alcohol; and Groups II, IV and V were given *Rhus tox.* in potencies of 6C, 30C and 200C, respectively. The study was carried out in two steps. In step 1, before dosing, rats were fasted overnight and then administered treatments orally in a single dose of 2000 µL/kg body weight. Food was withheld for another 3–4 h after administration. If no mortality was observed in these animals, the interventions were dosed in another set of three animals at 2000 µL/kg to confirm the same, that is, step 2. If test substance-related mortality occurred, further testing at the next lower level, that is, 1000 µL/kg in two steps, might have been required. During the experiment, the animals were individually observed for mortality and clinical signs of toxicity post-dosing, hourly for 8 h and then periodically for up to 14 days in both steps 1 and 2. The observations included physical changes (changes in fur, eyes and urine colour), behavioural (alertness, vocalisation, tremors, twitches, convulsions, sedation, sleep, catatonia and ataxia), respiratory pattern (apnoea and dyspnoea), reflexes, posture (body and limb positions) and autonomic effects (discoloured faeces, urination, lacrimation and piloerection). Body weight was measured weekly. At the end of the 14th day, all animals were sacrificed by carbon dioxide (CO₂) euthanasia, dissected and a gross necropsy examination was carried out.

**Sub-acute oral toxicity study**

Sub-acute oral toxicity study was carried out adhering to OECD guideline 407 methodology in male and female Wistar albino rats. 100 rats (50 males and 50 females) with body weights 150-200g were used in this study. First, 50 animals were randomly divided into five groups (five males and five females in each). Groups 1, 2 and 4 received *Rhus tox.* 30C, 6C and 200C, respectively. Group 3 (vehicle control) received 90% alcohol and Group 5 (control) received distilled water. In addition, recovery groups for control, vehicle control and *Rhus tox.* 6C, 30C and 200C were kept, each with ten animals (five males and five females) and given respective treatment for 28 days. They were monitored for a further recovery period of 14 days post-treatment to monitor reversibility, persistence, or delayed occurrence of toxic effects. All the animals received their respective treatments at a dose volume of 200 µL/kg, diluted with a 1:9 ratio of distilled water orally for 28 days.

During the treatment phase, general clinical signs were examined once daily, preferably 1 h after dosing, to capture the peak period of predicted effects following dosing. Daily physical and behavioural observations, appendages, posture, sensory responses, reflexes, respiratory and autonomic effects were monitored and recorded. Additional observations included mortality, body weight and feed consumption once a week, haematological parameters and clinical biochemistry parameters on the 29th and 43rd day, respectively. All surviving rats were euthanised on day 29 (control and treated groups) and day 43 (recovery groups) using CO₂ asphyxiation followed by exsanguination for gross pathology examination. Macroscopic
and histopathological examinations of tissues/organisms were
carried out for all groups.

Relative organ weight and % body weight changes were
calculated using the following equations:

\[
\frac{\text{Day 1} - \text{Day 14}}{\text{Day 1}} \times 100 \quad (1)
\]

Relative Organ Weight = \[
\frac{\text{Absolute Organ weight}}{\text{Body weight at sacrifice}} \times 100 \quad (2)
\]

**Blood collection and serum separation**

Rats fasted overnight before blood was drawn from the retro-
orbital sinus under ketamine and xylazine anaesthesia. The
blood was collected for haematology in potassium EDTA tubes
and biochemistry in clot activator tubes. The haematological
analysis was done using a veterinary haematology analyser.
The serum for clinical biochemistry analysis was separated by
centrifuging blood samples at 5000 rpm for 10 min at 25°C and
samples were analysed using a fully automated biochemistry
analyser with commercially available biochemistry kits
(Transasia Biomedicals. Ltd, Mumbai, India).

**Gross necropsy and histopathology**

After the completion of the study, rats were euthanised and subjected to a detailed gross necropsy and findings were recorded. The brain, thymus, heart, liver, lungs, adrenals, kidneys, spleen, testes/ovaries and uterus were isolated, adherent adipose tissues from the organs were trimmed off and relative organ weights were recorded. The tissues were preserved in 10% phosphate-buffered neutral formalin and then processed to make sections following the standard procedure.\(^{[18]}\) Followed by slides were stained with haematoxylin-eosin and histopathological inspection was carried out using an upright microscope.

**Statistical analysis**

Statistical analysis of the data was achieved by one-way analysis of variance followed by Tukey’s *post hoc* test using SPSS V26. Data were presented as mean ± standard error of mean. *P* < 0.05 was considered to be statistically significant.

**Results**

**Acute oral toxicity study**

No mortality was found in the animals who were administered either *Rhus tox*. single dose in 6C, 30C and 200C potencies, or the controls (alcohol/distilled water) in steps 1 and 2 [Supplementary Table 1]. In steps 1 and 2, transient ataxia was observed among the animals administered with *Rhus tox*. 6C, 30C, 200C and vehicle control, respectively, recovering within 3 h. Mild effects in limb position were observed among all groups of steps 1 and 2, except normal control, also recovering within 03 h. Marked effects in limb position in 2 animals treated with *Rhus tox*. 6C in step 1 disappeared within 24 h [Supplementary Table 1].

None of the treated animals exhibited any additional clinical signs or symptoms of toxicity and appeared to be healthy. No significant body weight changes were observed in animals during the study [Supplementary Table 2]. The treated animals from steps 1 and 2 had normal external body surfaces, orifices (such as the nasal, urethral, vaginal and anal) and cavities (such as the abdominal and thoracic) on gross necropsy evaluation. The macroscopical observation of tissues revealed
no abnormalities and hence no microscopical examination was carried out.

The results indicate that lethal dose 50 (L.D.50) should be >2000 µL/kg body weight as no mortality was observed in animals administered with *Rhus tox*.

**Sub-acute oral toxicity study**

**General clinical signs and mortality**

Rats from the *Rhus tox* treated and control groups did not show any abnormalities during the extensive clinical observations, which comprised home cage observations, handling observations, open field observations and sensory observations. No animal mortality was noticed during the study, including the recovery phase, proving *Rhus tox* in 6C, 30C and 200C potencies, was safe in subacute dose [Supplementary Table 3].

**Feed intake and body weight**

In the subacute toxicity experiment, no statistically significant changes in feed intake or absolute body weight were observed in either male or female animals receiving *Rhus tox* and vehicle control for 28 days compared to the normal control. This pattern also persisted during the recovery phase of the study [Figures 2-5].

**Biochemical and Haematological analysis**

In a subacute toxicity study of *Rhus tox*, biochemical analysis revealed that serum levels of marker enzymes did not differ significantly between male and female rat groups compared to the control group [Tables 1-3]. Similarly, in evaluating haematological parameters, no significant adverse treatment-related changes were observed in *Rhus tox* and dispensing alcohol-treated animals compared to their respective normal control groups [Supplementary Tables 4-6].

**Gross necropsy and histopathological evaluations**

Gross necropsy examination of rats treated with *Rhus tox* revealed normal external morphological features with no signs of abnormalities on external surfaces of tissues belonging to cardiovascular, urogenital, gastrointestinal, reproductive and central nervous systems. Compared to normal control rats, there were no treatment-related changes in the relative organ weights of the *Rhus tox* and dispensing alcohol-treated animals [Supplementary Tables 7 and 8]. In addition, histopathological examination of the organs (brain, thymus, heart, liver, lungs, adrenals, kidneys, spleen, testes, ovaries and uterus) revealed no pathological features in the control or *Rhus tox* treated groups [Figures 6 and 7]. Therefore, the study suggests that the 28 days of *Rhus tox* treatment did not cause significant adverse effects on the animals.

**Discussion**

The findings revealed that *Rhus tox* did not cause significant toxicity at the tested doses and the study provides evidence on the safety of *Rhus tox* in 6C, 30C and 200C potencies.
In the acute oral toxicity study, a single acute dose of Rhus tox. administered to Wistar albino rats at a dose volume of 2000 µL/kg could not cause any mortality, apart from a few clinical signs reversed in a short period. Transient ataxia and mild effects in limb position were observed in the animals treated with Rhus tox. 6C, 30C and 200C, and vehicle control. According to Novier et al., acute alcohol administration in young adult rats showed ataxia accelerating rotarod test, which improved within a period of 60 min. Hence, the ataxia and mild limb effects observed during the study can be considered as clinically insignificant and may be attributed to alcohol, that is, vehicle control. Therefore, the median LD₉₀ is determined to be >2000 µL/kg. Further, in the sub-acute toxicity study, Rhus tox. was evaluated at a dose volume of 200 µL/kg/day/rat diluted with a 1:9 ratio of distilled water, that is, 1/10th of 2000 µL/kg. Moreover, at this dose volume of 200 µL/kg diluted with a 1:9 ratio of distilled water, Rhus tox. in potencies was found to have efficacy in the previously published preclinical scientific reports on inflammatory and immune-related conditions.

Our study found that rats exposed to Rhus tox. showed normal behaviour and reactions in their cage. This suggests that Rhus tox. probably does not have any adverse effect on neurobehavioural. While there are no directly comparable studies on the neurophysiological effects of Rhus tox. in rodents, our findings align with findings of pre-clinical safety studies on plant derived homeopathic medicines, that is, Bellis perennis, Curcuma longa, Rauwolfia serpentina, Ricinus communis, Tribulus terrestris and Terminalia arjuna. When the body weight and relative organ weight of all the animals in the sub-acute study were statistically examined, no significant changes were found. This eliminates any potential treatment-related effects of Rhus tox. In addition, feed intake is crucial in assessing a drug’s safety for therapeutic use and increased body and organ weight.

It was clear that the appetite of the Rhus tox. treated animals was unaffected because there were no significant differences in the amount of feed they consumed, compared to the normal control group.

Orally administered medications are primarily delivered to the target through the blood, and both in humans and animals, blood cell loss or injury negatively impacts how well the body operates. Since the haematological system has a higher clinical relevance for toxicity, blood tests are essential for risk assessment. The haematological analysis showed that rats treated with Rhus tox. exhibited normal haematological profiles, and their values did not significantly differ from those of the control group, indicating that Rhus tox. does not have any detrimental effects on haematopoesis. When assessing the toxicity of medications, biochemical data are crucial. Since the liver and kidney are essential for an organism’s existence, their functions must be investigated to determine toxicity. The current investigation evaluated and analysed two serum renal markers, that is, serum creatinine and urea. Serum creatinine is the laboratory test most frequently used to assess renal function. Hepatic function was monitored by analysing SGOT, SGPT and ALP along with total and direct bilirubin. The administration of Rhus tox. in the current study did not result in significant changes in hepatic or renal biochemical parameters, confirming its safety when used repeatedly.

Blood glucose monitoring detects patterns in the fluctuations of blood sugar levels caused by factors such as alterations in diet, physical activity, medication and health conditions like diabetes, which are linked to shifts in blood glucose. Abnormal blood sugar levels can have immediate fatal consequences or long-term complications. Furthermore, the evaluation of lipid profiles should be included in the total risk assessment for cardiovascular or metabolic diseases. Therefore, the current study examined triglycerides, total cholesterol, HDL, LDL and fasting blood glucose. Rats treated with Rhus tox. had serum glucose and lipids levels within the typical clinical range throughout the research period, indicating no adverse effects on the body’s metabolic process during 28 days of administration.

Electrolytes are electrically charged molecules necessary for blood clotting, muscle and body fluid contractions and maintaining the right balance of the acid-base system. Their primary role is maintaining the fluid equilibrium between the intracellular and extracellular environments, which is essential for controlling pH, muscular contraction, nerve impulses and hydration. In the current study, Rhus tox. treatment caused no deviations in serum levels of sodium, potassium, chloride and calcium compared to the normal control group indicating no electrolyte imbalance during its administration. Histopathological analysis of tissues of rats treated with Rhus tox. further supports its safety, as no abnormal changes have been found. From the subacute toxicity study results, no observed adverse effect level of Rhus tox. 6C, 30C and 200C potencies are found to be greater than 200 µL/kg/day rat body weight.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Vehicle control</th>
<th>Rhus tox. - 6C</th>
<th>Rhus tox. - 30C</th>
<th>Rhus tox. - 200C</th>
<th>Control&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Vehicle control&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Rhus tox. - 6C&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Rhus tox. - 30C&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Rhus tox. - 200C&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Glucose (mg/dL)</td>
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<td>49.02±3.53</td>
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<td>0.39±0.00</td>
<td>0.39±0.02</td>
<td>0.37±0.04</td>
<td>0.38±0.04</td>
<td>0.34±0.03</td>
<td>0.38±0.01</td>
<td>0.38±0.01</td>
<td>0.36±0.02</td>
<td>0.34±0.02</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>10.38±0.15</td>
<td>10.24±0.11</td>
<td>10.24±0.10</td>
<td>10.14±0.07</td>
<td>10.46±0.24</td>
<td>10.26±0.13</td>
<td>10.16±0.07</td>
<td>10.26±0.10</td>
<td>10.28±0.10</td>
<td>10.36±0.15</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>142.58±0.16</td>
<td>142.22±0.27</td>
<td>142.10±0.28</td>
<td>142.48±0.17</td>
<td>142.90±0.21</td>
<td>142.74±0.19</td>
<td>142.30±0.07</td>
<td>142.32±0.32</td>
<td>142.32±0.22</td>
<td>142.72±0.26</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.40±0.09</td>
<td>4.29±0.04</td>
<td>4.32±0.07</td>
<td>4.38±0.12</td>
<td>4.74±0.20</td>
<td>4.23±0.10</td>
<td>4.35±0.04</td>
<td>4.33±0.06</td>
<td>4.44±0.12</td>
<td>4.32±0.10</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>102.32±0.53</td>
<td>101.88±0.36</td>
<td>101.56±0.25</td>
<td>101.66±0.35</td>
<td>102.02±0.48</td>
<td>101.76±0.38</td>
<td>101.68±0.46</td>
<td>101.94±0.39</td>
<td>101.84±0.39</td>
<td>101.30±0.34</td>
</tr>
</tbody>
</table>

Values expressed as mean±SEM. n=05. TP: Total protein, SGOT: Serum Glutamate Oxaloacetate Transferase, SGPT: Serum Glutamate Pyruvate Transferase, ALP: Alkaline phosphatase, TB: Total Bilirubin, DB: Direct Bilirubin, SC: Serum Creatine, TC: Total Cholesterol, Tgl: Triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein. There were no statistically significant differences in the biochemical parameters. <sup>a</sup>=Recovery groups
Table 2: Biochemical parameters in female rats during sub-acute oral toxicity study of *Rhus tox*. - day 29

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Vehicle control</th>
<th><em>Rhus tox</em>. -6C</th>
<th><em>Rhus tox</em>. -30C</th>
<th><em>Rhus tox</em>. -200C</th>
<th>Control(^\text{R})</th>
<th>Vehicle control(^\text{R})</th>
<th><em>Rhus tox</em>. -6C(^\text{R})</th>
<th><em>Rhus tox</em>. -30C(^\text{R})</th>
<th><em>Rhus tox</em>. -200C(^\text{R})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>75.10±2.46</td>
<td>79.78±4.53</td>
<td>75.46±3.73</td>
<td>78.28±4.34</td>
<td>77.00±4.07</td>
<td>78.84±5.37</td>
<td>80.26±4.48</td>
<td>77.50±4.42</td>
<td>79.2×±4.88</td>
<td>77.26±4.34</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>55.40±2.04</td>
<td>56.40±1.72</td>
<td>56.20±1.98</td>
<td>54.60±3.39</td>
<td>57.20±1.96</td>
<td>56.20±1.02</td>
<td>56.40±1.12</td>
<td>56.60±1.94</td>
<td>56.80±1.77</td>
<td>56.40±2.84</td>
</tr>
<tr>
<td>Tgl (mg/dL)</td>
<td>25.48±2.18</td>
<td>25.26±2.11</td>
<td>30.68±1.52</td>
<td>31.10±4.18</td>
<td>27.08±2.65</td>
<td>24.38±1.78</td>
<td>24.20±2.25</td>
<td>26.26±2.53</td>
<td>28.38±2.33</td>
<td>24.92±2.39</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>40.36±0.74</td>
<td>41.20±0.56</td>
<td>40.84±0.88</td>
<td>40.20±0.65</td>
<td>40.18±0.65</td>
<td>41.24±0.72</td>
<td>40.88±0.90</td>
<td>41.38±0.64</td>
<td>40.64±1.01</td>
<td>41.18±0.86</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>38.14±1.38</td>
<td>38.62±1.82</td>
<td>40.82±2.22</td>
<td>38.15±1.56</td>
<td>37.48±1.79</td>
<td>36.12±0.83</td>
<td>37.45±1.34</td>
<td>34.01±0.59</td>
<td>35.90±0.98</td>
<td>36.59±1.39</td>
</tr>
<tr>
<td>SGOT (U/L)</td>
<td>115.98±9.32</td>
<td>117.54±5.21</td>
<td>112.28±7.83</td>
<td>116.28±9.93</td>
<td>117.06±6.89</td>
<td>112.72±4.63</td>
<td>118.46±5.50</td>
<td>118.38±7.00</td>
<td>116.06±6.05</td>
<td>114.02±4.83</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>24.66±2.23</td>
<td>25.10±1.31</td>
<td>24.04±0.67</td>
<td>23.44±1.50</td>
<td>26.10±1.82</td>
<td>24.38±1.62</td>
<td>25.22±1.65</td>
<td>25.70±1.79</td>
<td>24.40±1.76</td>
<td>23.82±2.07</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>76.04±5.36</td>
<td>77.04±4.65</td>
<td>75.68±3.51</td>
<td>82.66±6.11</td>
<td>74.46±3.58</td>
<td>75.84±5.95</td>
<td>77.82±5.08</td>
<td>77.36±5.10</td>
<td>74.72±5.03</td>
<td>75.10±4.71</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.14±0.10</td>
<td>4.18±0.04</td>
<td>4.22±0.05</td>
<td>4.24±0.09</td>
<td>4.18±0.10</td>
<td>4.16±0.05</td>
<td>4.26±0.07</td>
<td>4.16±0.10</td>
<td>4.20±0.03</td>
<td>4.22±0.02</td>
</tr>
<tr>
<td>TP (g/dL)</td>
<td>6.78±0.12</td>
<td>6.60±0.13</td>
<td>6.84±0.13</td>
<td>6.48±0.13</td>
<td>6.82±0.20</td>
<td>6.62±0.17</td>
<td>6.64±0.13</td>
<td>6.52±0.09</td>
<td>6.58±0.18</td>
<td>6.68±0.10</td>
</tr>
<tr>
<td>TB (mg/dL)</td>
<td>0.09±0.00</td>
<td>0.09±0.00</td>
<td>0.09±0.00</td>
<td>0.08±0.00</td>
<td>0.09±0.00</td>
<td>0.08±0.01</td>
<td>0.08±0.01</td>
<td>0.09±0.00</td>
<td>0.08±0.00</td>
<td>0.08±0.00</td>
</tr>
<tr>
<td>DB (mg/dL)</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
<td>0.03±0.00</td>
<td>0.04±0.00</td>
<td>0.03±0.00</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
<td>0.03±0.00</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>21.80±1.16</td>
<td>21.74±1.41</td>
<td>20.96±1.37</td>
<td>20.62±1.51</td>
<td>21.54±1.37</td>
<td>22.04±1.72</td>
<td>21.72±1.14</td>
<td>22.04±0.97</td>
<td>21.80±1.95</td>
<td>21.70±1.15</td>
</tr>
<tr>
<td>SC (mg/dL)</td>
<td>0.39±0.01</td>
<td>0.40±0.01</td>
<td>0.40±0.01</td>
<td>0.37±0.01</td>
<td>0.41±0.01</td>
<td>0.40±0.00</td>
<td>0.41±0.01</td>
<td>0.40±0.01</td>
<td>0.40±0.01</td>
<td>0.41±0.01</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>10.18±0.10</td>
<td>10.20±0.10</td>
<td>10.16±0.04</td>
<td>10.30±0.15</td>
<td>10.22±0.07</td>
<td>10.20±0.08</td>
<td>10.20±0.08</td>
<td>10.22±0.08</td>
<td>10.16±0.11</td>
<td>10.18±0.11</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>143.80±0.34</td>
<td>144.02±0.12</td>
<td>144.08±0.17</td>
<td>143.68±0.17</td>
<td>144.12±0.17</td>
<td>143.52±0.14</td>
<td>144.04±0.16</td>
<td>143.30±0.27</td>
<td>143.54±0.22</td>
<td>144.02±0.16</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.07±0.12</td>
<td>4.39±0.07</td>
<td>4.21±0.07</td>
<td>4.14±0.07</td>
<td>4.26±0.05</td>
<td>4.17±0.10</td>
<td>4.30±0.12</td>
<td>4.26±0.12</td>
<td>4.19±0.09</td>
<td>4.30±0.17</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>103.26±0.08</td>
<td>103.56±0.16</td>
<td>103.26±0.07</td>
<td>103.46±0.22</td>
<td>103.38±0.16</td>
<td>103.44±0.16</td>
<td>103.66±0.13</td>
<td>103.66±0.23</td>
<td>103.52±0.32</td>
<td>103.86±0.14</td>
</tr>
</tbody>
</table>

Values expressed as mean±SEM. \(n=05\). TP: Total protein, SGOT: Serum Glutamate Oxaloacetate Transf erase, SGPT: Serum Glutamate Pyruvate Transf erase, ALP: Alkaline phosphatase, TB: Total Bilirubin, DB: Direct Bilirubin, SC: Serum Creatinine, TC: Total Cholesterol, Tgl: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein. There were no statistically significant differences in the biochemical parameters. \(^\text{R}\): Recovery groups.
Table 3: Biochemical parameters in rats during sub-acute oral toxicity study of *Rhus tox*. - day 43 (Recovery period)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Vehicle Control&lt;sup&gt;a&lt;/sup&gt;</th>
<th><em>Rhus tox</em>. -6&lt;sup&gt;b&lt;/sup&gt;</th>
<th><em>Rhus tox</em>. -30&lt;sup&gt;c&lt;/sup&gt;</th>
<th><em>Rhus tox</em>. -200&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Control&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Vehicle Control&lt;sup&gt;a&lt;/sup&gt;</th>
<th><em>Rhus tox</em>. -6&lt;sup&gt;b&lt;/sup&gt;</th>
<th><em>Rhus tox</em>. -30&lt;sup&gt;c&lt;/sup&gt;</th>
<th><em>Rhus tox</em>. -200&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>75.94±3.96</td>
<td>76.64±5.54</td>
<td>77.74±5.09</td>
<td>80.04±4.48</td>
<td>72.44±1.72</td>
<td>79.02±8.37</td>
<td>82.24±4.09</td>
<td>75.08±3.56</td>
<td>82.54±3.77</td>
<td>82.24±4.09</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>55.60±2.89</td>
<td>56.20±2.20</td>
<td>54.60±3.22</td>
<td>56.80±2.62</td>
<td>55.20±2.73</td>
<td>56.60±3.97</td>
<td>54.80±3.31</td>
<td>55.40±2.11</td>
<td>56.00±2.10</td>
<td>56.40±1.40</td>
</tr>
<tr>
<td>Tgl (mg/dL)</td>
<td>41.00±0.62</td>
<td>41.92±0.62</td>
<td>41.76±0.82</td>
<td>41.74±0.68</td>
<td>41.28±0.78</td>
<td>40.56±1.31</td>
<td>41.20±0.77</td>
<td>41.02±0.57</td>
<td>40.68±1.09</td>
<td>40.50±0.95</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>34.86±0.85</td>
<td>37.59±1.23</td>
<td>37.16±1.77</td>
<td>36.32±1.29</td>
<td>34.42±0.76</td>
<td>35.42±0.69</td>
<td>37.68±1.29</td>
<td>34.47±0.62</td>
<td>37.33±0.89</td>
<td>36.34±1.79</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>26.46±2.15</td>
<td>27.88±2.11</td>
<td>27.06±2.01</td>
<td>30.42±1.82</td>
<td>25.82±2.33</td>
<td>24.18±1.71</td>
<td>22.62±2.52</td>
<td>25.14±1.90</td>
<td>24.66±1.42</td>
<td>25.20±1.09</td>
</tr>
<tr>
<td>SC (mg/dL)</td>
<td>114.48±6.83</td>
<td>112.54±8.46</td>
<td>113.20±7.59</td>
<td>112.8±9.92</td>
<td>111.84±8.46</td>
<td>76.54±6.61</td>
<td>73.54±5.20</td>
<td>78.22±5.31</td>
<td>76.20±5.77</td>
<td>78.02±4.70</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.92±0.10</td>
<td>4.02±0.07</td>
<td>3.98±0.12</td>
<td>3.88±0.10</td>
<td>4.04±0.08</td>
<td>4.22±0.02</td>
<td>4.20±0.05</td>
<td>4.22±0.10</td>
<td>4.10±0.04</td>
<td>4.20±0.03</td>
</tr>
<tr>
<td>TP (g/dL)</td>
<td>6.30±0.10</td>
<td>6.26±0.07</td>
<td>6.28±0.10</td>
<td>6.34±0.09</td>
<td>6.14±0.14</td>
<td>6.56±0.16</td>
<td>6.62±0.12</td>
<td>6.52±0.12</td>
<td>6.64±0.16</td>
<td>6.62±0.15</td>
</tr>
<tr>
<td>TB (mg/dL)</td>
<td>0.08±0.00</td>
<td>0.08±0.00</td>
<td>0.08±0.00</td>
<td>0.08±0.00</td>
<td>0.07±0.00</td>
<td>0.09±0.01</td>
<td>0.08±0.00</td>
<td>0.08±0.00</td>
<td>0.08±0.00</td>
<td>0.09±0.00</td>
</tr>
<tr>
<td>DB (mg/dL)</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
<td>0.03±0.01</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>17.36±0.19</td>
<td>16.80±0.22</td>
<td>17.02±0.26</td>
<td>17.00±0.10</td>
<td>17.16±0.25</td>
<td>22.50±4.23</td>
<td>21.24±1.39</td>
<td>21.20±1.30</td>
<td>21.54±1.34</td>
<td>21.34±1.71</td>
</tr>
<tr>
<td>SC (mg/dL)</td>
<td>0.37±0.01</td>
<td>0.39±0.01</td>
<td>0.38±0.01</td>
<td>0.38±0.02</td>
<td>0.38±0.02</td>
<td>0.41±0.02</td>
<td>0.41±0.01</td>
<td>0.40±0.00</td>
<td>0.40±0.01</td>
<td>0.41±0.00</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>10.18±0.05</td>
<td>10.03±0.05</td>
<td>10.18±0.04</td>
<td>10.26±0.06</td>
<td>10.80±0.05</td>
<td>10.08±0.08</td>
<td>10.20±0.09</td>
<td>10.18±0.09</td>
<td>10.20±0.08</td>
<td>10.16±0.10</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>141.76±0.27</td>
<td>141.90±0.19</td>
<td>142.38±0.24</td>
<td>141.78±0.31</td>
<td>142.02±0.17</td>
<td>143.58±0.23</td>
<td>143.90±0.27</td>
<td>143.56±0.26</td>
<td>143.58±0.28</td>
<td>143.82±0.19</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.41±0.09</td>
<td>4.31±0.09</td>
<td>4.35±0.10</td>
<td>4.42±0.10</td>
<td>4.32±0.08</td>
<td>4.14±0.18</td>
<td>4.19±0.16</td>
<td>4.26±0.14</td>
<td>4.14±0.13</td>
<td>4.30±0.09</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>101.32±0.29</td>
<td>101.10±0.16</td>
<td>101.46±0.35</td>
<td>101.70±0.44</td>
<td>101.06±0.33</td>
<td>103.50±0.17</td>
<td>103.74±0.22</td>
<td>103.40±0.30</td>
<td>103.46±0.34</td>
<td>103.48±0.16</td>
</tr>
</tbody>
</table>
| Values expressed as mean±SEM. *n*=05. TP: Total protein, SGOT: Serum Glutamate Oxaloacetate Transferase, SGPT: Serum Glutamate Pyruvate Transferase, ALP: Alkaline phosphatase, TB: Total Bilirubin, DB: Direct Bilirubin, SC: Serum Creatinine, TC: Total Cholesterol, Tgl: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein. There were no statistically significant differences in the biochemical parameters. <sup>b</sup>: Recovery groups
Conclusion

The present study suggests that *Rhus toxicodendron* in 6C, 30C and 200C potencies is safe on repeated administration and does not alter the treated animals’ cellular, biochemical and physiological ecosystems. The study provides a foundation for future research on the safety and efficacy of *Rhus toxicodendron* in clinical trials employing humans.

Conflict of interest

None.

Financial support

Central Council for Research in Homoeopathy, India, sponsored the study under Intramural Research Scheme. (Grant no: F.No- 4-22/2019-20/CCRH/Tech/DS/(DS Annual Assignment)/1662 dated 16-10-2020).
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Supplementary Tables
Supplementary Table 1: Cumulative record for clinical signs and mortality in rats treated with Rhus tox. during acute oral toxicity study (n=3)
Supplementary Table 2: Body weight (g) measurements during acute oral toxicity study of Rhus tox.
Supplementary Table 3: Mortality in rats treated with Rhus tox. during subacute oral toxicity study (n=5)
Supplementary Table 4: Haematological parameters in male rats during sub-acute oral toxicity study of Rhus tox. - day 29
Supplementary Table 5: Haematological parameters in female rats during sub-acute oral toxicity study of Rhus tox. - day 29
Supplementary Table 6: Haematological parameters in male rats during sub-acute oral toxicity study of Rhus tox. - day 43 (Recovery period)
Supplementary Table 7: Effect of Rhus tox. on Relative Organ Weights in percentage of male rats – (Treatment+Recovery period)
Supplementary Table 8: Effect of Rhus tox. on relative organ weights in percentage of female rats – (Treatment+Recovery period)

References
2. NCIRS. Homoeopathy and Vaccination. NCIRS Fact Sheet; 2014.
Title: Experimenteller Nachweis der Sicherheit von potenzierten Verdünnungen von Rhus toxicodendron bei Wistar-Albino-Ratten

Título: Evidencia experimental de la seguridad de diluciones potenciadas de Rhus toxicodendron en ratas albinas Wistar

Antecedentes: Rhus toxicodendron (Rhus tox.) es un medicamento homeopático bien conocido que se utiliza para tratar trastornos inflamatorios respaldado por evidencia científica histórica y moderna. Objetivo: La seguridad de las potencias 6C, 30C y 200C de Rhus tox. a través de pruebas de toxicidad oral aguda y subaguda según las pautas de prueba de la Organización para la Cooperación y el Desarrollo Económico. Métodos: Para el estudio de toxicidad oral aguda, Rhus tox. (2000 µL/kg) a ratas y se observaron durante 14 días. Para el estudio de toxicidad oral subaguda, Rhus tox. (200 µL/kg) durante 28 días y se incluyeron grupos de recuperación adicionales para monitorear la reversibilidad, persistencia o aparición retardada de efectos tóxicos durante 14 días después del tratamiento. Se realizaron evaluaciones histopatológicas de órganos vitales para detectar cualquier signo de toxicidad. Resultados: No se produjo mortalidad durante el estudio de toxicidad aguda a una dosis de 2000 µL/kg, lo que indica una DL50 oral de Rhus tox. > 2000 µL/kg. En el estudio de toxicidad subaguda, Rhus tox. La administración durante 28 días no mostró signos clínicos adversos, con aumento de peso y consumo de alimento normales en los animales tratados. No se observaron cambios adversos en los parámetros bioquímicos y hematológicos de Rhus tox. ratas tratadas. Además, no se observaron anomalías en los exámenes macroscópicos e histopatológicos de órganos vitales. Conclusión: El estudio encontró que Rhus tox. en potencias de 6C, 30C y 200C exhibió un perfil toxicológico seguro, lo que respalda sus efectos farmacológicos beneficiosos.