Comparative evaluation of the effects of n-hexane, chloroform, and methanol fractions of *Ricinus communis* in carbon tetrachloride-induced hepatotoxic rats

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**ABSTRACT**

**Background:** *Ricinus communis* is widely used among the Nigerian populace in ethnomedicine for the treatment of liver diseases. **Aim of the Study:** A comparative study was carried out to evaluate the hepatoprotective activity of its n-hexane, chloroform, and methanol fractions using carbon tetrachloride-(CCL₄)-induced hepatotoxicity model. **Materials and Methods:** Thirty Wistar rats were randomized as follows: Negative control Group 1 received Tween 80 and positive Group 2 received CCL₄ only. Treatment Groups (3, 4, and 5) received CCl₄ + N-hexane, chloroform, and methanol fractions, 100 mg/kg, respectively. Standard reference, Group 6, received CCl₄ + Silymarin, 100 mg/kg. The animals were treated for 7 consecutive days and hepatotoxicity was induced in Groups 1, 2, 3, 4, and 6 by a single oral administration of CCL₄ (2 mL/kg). Thereafter, blood samples were collected for assay of hematological parameters and liver enzymes (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]). **Results:** There were significant decrease (*P* < 0.05) in serum AST/ALT levels of animals treated with chloroform fraction (50.00 ± 6.01 µ/L/21.40 ± 0.98 µ/L) and methanol fraction (51.25 ± 2.88 µ/L/24.80 ± 1.52 µ/L). The chloroform fraction produced a better improvement in hematological parameters when compared with other treatment groups. Photomicrograph showed near-normal structural liver integrity of the rats administered chloroform fraction. **Conclusions:** This study validates the usefulness of *R. communis* in the treatment of hematological disorders in patients with hepatic disorders. The chloroform fraction could be a promising fraction for further investigation and characterization of hepatoprotective compound(s).

**Keywords:** Carbon tetrachloride, hematology, hepatoprotection, *Ricinus communis*, rodents

**INTRODUCTION**

The liver is a vital organ involved in extramedullary hematopoiesis, the synthesis of transferrin (a carrier protein that transports iron for erythropoiesis), coagulation factors, storage of Vitamin B12, folic acid, and iron as well as the destruction and removal of red cells.¹ ² Although the liver has a high regenerative capacity, the numerous roles of the organ, especially detoxification and metabolism, can overwhelm its recovery from injury.

Chemical-induced hepatic damage is becoming overwhelming due to exposure of subjects to environmental and industrial chemicals as well as food and drug poisoning. Furthermore, its metabolic function puts the liver at a strategic disadvantage of injury, especially of toxicants triggered by metabolism through hepatic enzymes. Compromised liver function following hepatic injury could render the body system prone to serious health complications as a result of immune suppression.³ ⁴ Chronic liver diseases, in most cases, are associated with hematological disorders. Anemia has been shown to occur in 75% of patients with chronic liver diseases.³ ⁴ Studies have shown that carbon tetrachloride (CCL₄) toxicity caused anemia as well as impairment in red blood cell (RBC) production.³ ⁵

In the management of liver disorders, no ideal synthetic hepatoprotective agents have been discovered because...
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some of them have limitations in their use. In light of this, drug discovery/development studies including alternative approaches in the form of herbal medicine are being pursued. To this end, a number of medicinal plants such as *Pueraria lobata* and *Withania somnifera* and isolated molecules such as silymarin have been reported to possess hepatoprotective activity.[6,7]

*Ricinus communis*, popularly known as castor oil plant, belongs to the family Euphorbiaceae, an evergreen shrub growing up to 5 m tall that is widespread in tropical areas. A recent review by Manoj[8] revealed that it possesses several medicinal values which have been validated through pharmacological screening. Among these include antiulcer,[9] antioxidant,[10] antidiabetic,[11] as well as hepatoprotective activities.[12,14] Phytochemical evaluation of *R. communis* revealed the presence of anthraquinones, alkaloids, phobatanin, flavonoids, saponins, steroids, and glycosides.[13]

In an attempt to identify the most promising fractions which could be of usefulness in drug development, the present study evaluated the effect of n-hexane, methanol, and chloroform fractions of *R. communis* using hematological and liver enzymes as biomarkers of hepatic function in CCL$_4$ model in rats.

**MATERIALS AND METHODS**

**Preparation of Plant Fraction**

Fresh leaf of *R. communis* was collected from the wild in Delta State, Nigeria. The plant was authenticated with a voucher deposit (FHI 108864) at the Forestry Research Institute, Nigeria. The leaf of *R. communis* was cleaned with water, dried at room temperature and pulverized. A total of 1.8 kg of the powdered leaves was cold-extracted serially with a known volume of n-hexane, chloroform, and methanol, consecutively. At each stage, the filtrate was concentrated with a rotary evaporator to obtain the n-hexane, chloroform, and methanol fractions, respectively. The percentage yield for each fraction was calculated. The fractions were reconstituted in Tween-80 before the administration to the laboratory animals; the concentrated fraction could not dissolve in water. The doses of the fractions were determined following a pilot study and literature search.[14]

**Animals**

A total of 30 healthy albino rats used for this study were procured from the Animal House of the Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria. They were given access to rat chow and water ad libitum. Animals were allowed to acclimatize for 14 days before the commencement of the study. All experimental procedures were consistent with the NIH guidelines for the use and care of laboratory animals, as approved by the Ethics Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria (FBMS/234/8102).

**Induction of Hepatotoxicity**

Experimental animals were randomly allotted into six groups of five animals each. After fasting the animals for 12 h, hepatotoxicity was induced by a single oral administration of CCL$_4$ (2 mL/kg) to the experimental animals.[17] N-hexane, chloroform, and methanol fractions of *R. communis* (100 mg/kg) were administered to Groups 1, 2, and 3, respectively. Group 4 received Silymarin (100 mg/kg). Groups 5 and 6 served as CCL$_4$ and normal control (Tween 80), respectively. Treatment was done orally, once daily and it lasted 7 days.

**Sample Collection**

The animals were fasted for 12 h after the 7 days treatment. Blood samples were collected from the rats for hematological and liver biomarkers assay. The rats were then sacrificed and the liver harvested for histomorphological examinations.

**Assay of Hematological and Liver Biomarkers**

Hematological parameters, hemoglobin, hematocrit packed cell volume (PCV), RBC, platelet counts, white blood cell (WBC), and differentials (neutrophils and lymphocytes) were determined using the method described by Baker et al.[17] Liver enzymes, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined using the method of Reitman and Frankel.[18]

**Data Analysis**

Results are presented as mean ± standard deviation. Significant difference among groups was determined by one-way analysis of variance, using Statistical Package for the Social Science (SPSS-20, for windows). $P < 0.05$ was considered to be statistically significant.

**RESULTS**

The percentage yield for each of the fractions is presented as follows:

- N-hexane: $60/1800 \times 100/1 = 3.33\%$
- Chloroform: $76/1800 \times 100/1 = 4.22\%$
- Methanol: $76/1800 \times 100/1 = 4.22\%$

Results [Table 1] showed that there was a significant decrease ($P < 0.05$) in serum AST level of animals that were treated with chloroform fraction ($50.00 \pm 6.01 \mu L$), methanol fraction ($51.25 \pm 2.88 \mu L$) and silymarin ($49.60 \pm 8.38 \mu L$) when compared with AST level of animals in positive control ($75.23 \pm 5.06 \mu L$). In the same vein, serum ALT levels were observed to be significantly ($P < 0.05$) reduced following treatment with chloroform fraction ($21.40 \pm 0.98 \mu L$), methanol fraction ($24.80 \pm 1.52 \mu L$), and silymarin ($22.62 \pm 0.43 \mu L$) when compared with ALT level of animals in positive control ($40.33 \pm 1.50 \mu L$). The levels of the liver enzymes were similar in the animals administered standard agent (silymarin) and chloroform/methanol fractions. The same trend was also observed when the test groups administered chloroform/methanol fractions were compared with the negative control groups (normal animals administered Tween 80 only).

It was observed that administration of the fractions and silymarin caused a significant ($P < 0.05$) increase in PCV, platelet, WBC, neutrophils, and lymphocytes levels.
when compared with the untreated group (positive control) [Table 2]. A similar trend was also observed in RBC and hemoglobin, except for methanol fraction which did not produce any observable difference in PCV as well as n-hexane fraction which did not produce any observable difference in hemoglobin when compared with the negative control. Overall, the chloroform fraction produced a better improvement in hematological parameters when compared with other treatment groups.

Photomicrographs of sections of the liver of the experimental animals are shown in Figure 1a-f. Worthy of mention is near-normal structural integrity of sections of the liver of the rats administered silymarin [Figure 1c] and chloroform fraction [Figure 1f] of the test agents (R. communis) compared with that of the rats in the normal control group [Figure 1a] administered the vehicle (Tween-80). Comparatively, the protective effect of the aforementioned fractions is also visible against the effect of the hepatotoxic agents (CCL<sub>4</sub>), namely, multiple foci of hepatic necrosis and fatty infiltration and severe portal congestion, in the group administered CCL<sub>4</sub> only [Figure 1b].

**DISCUSSION**

The present study evaluated the hepatoprotective potentials of three fractions of R. communis leaf utilizing CCL<sub>4</sub> model of hepatotoxicity in rats. The CCL<sub>4</sub>-induced hepatic damage is an established model of investigating the hepatoprotective effects of drugs undergoing screening. The substance has been known to produce toxic effects on the liver through its conversion to a trichloromethyl free radical by the hepatic CYP<sub>450</sub> microsomal enzymes. The chain of reactive free radicals generated causes lipid peroxidation, damage of proteins, and other cell components.[19] This is also characterized by an elevation in the liver biomarkers, ALT, and AST; among others. [3] ALT and AST are largely used as biomarkers of xenobiotic-induced liver damage, which is characterized by elevated AST and ALT level as a result of a disruption in the integrity of hepatocytes plasma membrane and loss of their content into the blood. [20] The observed increase in ALT and AST, in the present study, is similar to the findings of Saba et al.[21] From this study, a significant elevation in ALT and AST levels observed following the administration of CCL<sub>4</sub>[21] was reduced after treatment with chloroform and methanol fractions, suggesting that the constituents in these fractions could be hepatoprotective. This finding is supported by the previous studies on the hepatoprotective effect of R. communis due to its ability to inhibit lipid peroxidation in the liver as well as the activities of elevated liver enzymes. [13,14] The protective effect of the chloroform fraction was further buttressed by the near-normal structural integrity of the liver tissues of the rats administered this test agent. This is in line with the findings of Shukla et al.[13] who reported increase viability of hepatocyte and near-normal restoration of enzymatic levels.

Administration of CCL<sub>4</sub> reported by a previous study[23] to cause derangement of hematological parameters was confirmed by the observation in the present study. Saba et al. reported deranged hematological parameters culminating in anemia.[20] In this study, the administration of R. communis produced a significant reduction in the effects of CCL<sub>4</sub> on hematological parameters. These effects may be attributed to the protective and reparative effect of the bioactive components of the fractions on the liver thereby preserving its functions which include the synthesis of transferrin and coagulation factors. A better effect produced by the chloroform fraction when compared with other groups could be attributed to a better absorption of its active components.

**Table 1:** Effect of fractions of *Ricinus communis* on AST and ALT levels of CCL<sub>4</sub> induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum AST (µ/L)</th>
<th>Serum ALT (µ/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Negative control (Twee 80)</td>
<td>48.37±1.67*</td>
<td>20.62±0.43*</td>
</tr>
<tr>
<td>Group 2</td>
<td>Positive control (CCL&lt;sub&gt;4&lt;/sub&gt; only)</td>
<td>75.23±5.06bc</td>
<td>40.33±1.50bc</td>
</tr>
<tr>
<td>Group 3</td>
<td>CCL&lt;sub&gt;4&lt;/sub&gt;+n-hexane fraction</td>
<td>71.19±7.78bc</td>
<td>32.40±1.49bc</td>
</tr>
<tr>
<td>Group 4</td>
<td>CCL&lt;sub&gt;4&lt;/sub&gt;+chloroform fraction</td>
<td>50.00±6.01</td>
<td>21.40±0.98</td>
</tr>
<tr>
<td>Group 5</td>
<td>CCL&lt;sub&gt;4&lt;/sub&gt;+methanol fraction</td>
<td>51.25±2.88*</td>
<td>24.80±1.52*</td>
</tr>
<tr>
<td>Group 6</td>
<td>Standard control (CCL&lt;sub&gt;4&lt;/sub&gt;+silymarin)</td>
<td>49.60±8.38*</td>
<td>22.62±0.43*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±standard deviation, n=5. *P<0.05: Statistically significantly different from CCL<sub>4</sub> control (positive control), Twee 80 (negative control), and silymarin (standard control), respectively. CCL<sub>4</sub>: Carbon tetrachloride, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

**Table 2:** Effect of fraction s of *Ricinus communis* on hematological parameters of carbon tetrachloride induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Packed cell volume (%)</th>
<th>Hemoglobin (g/dl)</th>
<th>Red blood cell×10&lt;sup&gt;12&lt;/sup&gt;/µL</th>
<th>Platelet×10&lt;sup&gt;12&lt;/sup&gt;/µL</th>
<th>White blood cell×10&lt;sup&gt;9&lt;/sup&gt;/µL</th>
<th>Neutrophils (%)</th>
<th>Lymphocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>47.20±3.96*</td>
<td>14.26±1.19*</td>
<td>5.64±0.56*</td>
<td>308.40±20.14*</td>
<td>8.84±2.69*</td>
<td>12.40±2.07*</td>
<td>77.60±6.47*</td>
</tr>
<tr>
<td>Group 2</td>
<td>47.25±3.99*</td>
<td>11.74±0.50*</td>
<td>4.49±0.19*</td>
<td>118.40±14.40*</td>
<td>4.68±0.72*</td>
<td>3.68±0.09*</td>
<td>50.70±0.30*</td>
</tr>
<tr>
<td>Group 3</td>
<td>42.25±0.50*</td>
<td>12.78±0.86*</td>
<td>4.58±0.56*</td>
<td>210.50±14.20*</td>
<td>6.50±0.79*</td>
<td>10.25±0.50*</td>
<td>79.75±0.50*</td>
</tr>
<tr>
<td>Group 4</td>
<td>46.67±2.31*</td>
<td>14.50±0.69*</td>
<td>5.27±0.25*</td>
<td>294.33±12.50*</td>
<td>7.63±1.53*</td>
<td>12.00±1.00*</td>
<td>82.00±7.00*</td>
</tr>
<tr>
<td>Group 5</td>
<td>43.33±1.53*</td>
<td>11.80±1.48*</td>
<td>5.07±0.21*</td>
<td>245.33±21.39*</td>
<td>7.20±1.37*</td>
<td>11.67±2.89*</td>
<td>70.00±8.66*</td>
</tr>
<tr>
<td>Group 6</td>
<td>46.80±2.68*</td>
<td>14.14±0.80*</td>
<td>5.50±0.64*</td>
<td>291.80±20.84*</td>
<td>7.78±3.14*</td>
<td>11.80±2.28*</td>
<td>80.20±2.28*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±standard deviation, n=5. *P<0.05: Statistically significantly different from Group 2 (positive control), Group 1 (negative control), and Group 6 (standard)
to the possibility of chloroform as a solvent to fraction more of the phytochemical compound(s) responsible for the effect on the deranged hematological parameters. It could be inferred, therefore, that the active component was isolated by and located in the chloroform fraction. Studies have shown that chloroform has the ability to fraction some secondary metabolites with biological activity.22,23 Going by its being a mid-polar organic solvent, chloroform characteristically dissolves both non-polar and some moderately polar molecules. In light of this, the active hepatoprotective compounds in R. communis may be non-polar to moderate polarity.

However, it has been reported that secondary metabolites of medicinal plants are responsible for their biological activities.14 Thus, the presence of bioactive phytochemicals such as flavonoids and tannins in R. communis may also be responsible for the regenerative and reparative capacity of the rat liver during hepatic injury caused by CCL₄.

CONCLUSIONS

This study demonstrates that the chloroform fraction of R. communis improved CCL₄ altered liver and hematological parameters. This finding provides preliminary data which may support the use of R. communis in the treatment of hematological disorders in patients with hepatic damage or impairment. Further studies to elucidate the bioactive constituents responsible for the hepatoprotective activity of the chloroform fraction are hereby recommended. The active metabolites of R. communis responsible for maintaining the structural and functional integrity of the liver may be located in the chloroform fraction.

REFERENCES