Acute toxicity, hepato-curative activity of extracts of a combination of plants on CCL4-induced hepatotoxicity in rats and antiradical activity

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Abstract

Traditional healers combine four medicinal plants (Cochlospermum tinctorium, Terminalia macroptera, Leptadenia hastata and Commiphora Africana) to treat hepatitis in Burkina Faso. The aimed was to evaluate the hepato-curative activity of lyophilized aqueous decoction (LAD) and hydroethanolic macerate (LHM) of plant extracts on CCl4-induced hepatitis in rats. We assessed the acute toxicity and scavenging activity of the 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). Hepato-curative activity study included nine groups with five rats each. We used rats as followed: group 1 as neutral controls, group 2 as negative controls, and the other groups were experimental groups. Rats in groups 2-9 received a single dose (1 mL/kg) of CCl4 in intraperitoneal injection to induce hepatitis. We fed orally the rats for seven consecutive days with sylimarin in group 3, LAD and LHM respectively in groups 4-6 and groups 7-9 by 400, 200 and 100 mg/kg/day. This study revealed LAD and LHM had a LD50 > 2000 mg/kg and both showed radical-scavenging properties with IC50 = 5.95 and 8.66 µg/mL respectively. All experimental rats regardless of the treatment group showed a significantly reduced plasma transaminases level as compared to negative controls. LAD and LHM at 400, 200 mg/kg significantly reduced alkaline phosphatase and gamma-glutamyl transferase. Histologically, treated rats showed normal to almost normal liver in a dose-dependent manner as compared to the controls.

Conclusion: LAD and LHM decreased liver enzyme and allowed a dose-Dependent liver damage recovery after CCl4-induced hepatitis in rats.

Keywords: Carbon tetrachloride; Medicinal plants; Hepato-curative; Liver enzymes; Burkina Faso

1. Introduction

Hepatitis, an inflammation of the liver, is usually associated with the use of various drugs, chemicals and viruses [1]. Viral hepatitis is a major public health problem worldwide and hepatitis B and C account for 96% of all hepatitis mortality. In 2015, hepatitis B alone was responsible for 1.34 million deaths [2]. Carbon tetrachloride (CCL4) is well-known to be hepatotoxic with similar liver damage seen in viral hepatitis, which justify its use in experimental animal model to screen for hepatoprotective and hepato-curative agents [3]. Cytochrome P450 metabolized CCL4 into free
In Burkina Faso, four medicinal plants have separately been used to treat liver conditions. Roots of *Cochlospermum tinctorium* Perrier ex A. Rich. (Bixaceae) has been used to treat liver disorders, cough, anemia; leaves of *Terminalia macroperata* (Guill. & Perr.) (Combretaceae) to treat liver disorders and ulcers; aerial parts of *Leptadenia hastata* Vatke (Apocynaceae) and roots of *Commiphora africana* (A. Rich.) Endl. (Burseraceae) have been used to treat jaundice, cough and fever. A mixture of these four medicinal plants has been used to treat hepatitis [5, 6].

Hence, we aimed to evaluate hepato-curative activity of the LAD and LHM extracted of this mixture on CCl₄-induced hepatotoxicity in rats.

### 2. Material and methods

#### 2.1. Reagents

We purchased Carbon Tetrachloride (CCl₄, 99%) from MERCK-Schuchardt (Germany) and ether diacetate from Sigma-Aldrich (Germany). We obtained Silymarin (Cefasilymarin®) from Cefak (Kempten), olive oil from Belle France (France) and formalin from Cooper. All these chemicals were analytical.

#### 2.2. Animals

We used 10-week-old male and female wistar rats weighing between 180 and 250 g from the International Center for Research-Development on Livestock in the Subhumid Zone (CIRDES) of Bobo-Dioulasso. We divided the rats into groups in cages lined with shavings and kept at 22-25°C with a 12-hour light/dark cycle. We fed rats with freely accessible granules, and tap water.

#### 2.3. Plants

We used aerial parts of *Leptadenia hastata* Vatke (Apocynaceae), leaves of *Terminalia macroperata* Guill. & Perr. (Combretaceae), roots of *Cochlospermum tinctorium* Perrier ex A. Rich. (Bixaceae) and *Commiphora africana* (A.Rich.) Endl. (Burseraceae). We harvested plants between 6 AM and 9 AM in July 2019 in Koubri, 29.6 km from Ouagadougou. A botanist from the Department of Traditional Medicine of Mali has authenticated each harvested plant and a herbarium of each plant was labeled as #1481, #2468, #0048, and #0076. We cleaned and dried part of plants in free air in the shade for two weeks and then grounded each part separately with Heto Drywinner®. We made a mixture with equal amount from each of the four plants. We mixed and stored the powders far from light and dust.

#### 2.4. Preparation of aqueous and hydroethanolic extracts

The extraction was performed following the method described previously with few adjustments [7]. To prepare the aqueous decoction, we mixed 30 g of powders with 300 mL distilled water, boiled the mixture for 15 minutes, let it cooled down and then filtered through a gaze and cotton. For the hydroethanolic maceration, we followed the same procedure except we used hydroethanolic solution 80% (20: 80 v/v water: ethanol) under magnetic agitation for 24 hours at room temperature before filtering on gaze and cotton.

We concentrated with a rotavapor, froze and freeze-dried the filtrates. We kept lyophilizates in sterile and hermetically sealed vials for future testing. The yield was 15.17% for LHM and 8.97% for LAD.

#### 2.5. Characterization of chemical constituents

We used chemical reaction characterization on tube and by thin layer chromatography (TLC) to identify chemical groups. We used Butanol-Acetic Acid-Water (60:15:25) and Ethyl Acetate-Methyl Ethyl Ketone-Formic Acid-Water (50:30:10:10) as solvents. The TLC plates were revealed by Godin’s reagent and iron trichloride (FeCl₃).

#### 2.6. Qualitative antioxidant activity on TLC plates

Ten (10) mg of either LAD or LHM was dissolved in 1 mL of methanol: water mixture (1:1). We then applied 10 µL of these solutions on Aluminum plate coated with silica gel 60 FG254 Merck support. We eluted TLC plate in Acetate-Methyl Ethyl Ketone-Formic Acid-Water (50: 30: 10: 10), dried it up and then sprayed it with a 1,1-diphenyl-2-picryl-hydrazyl (DPPH) solution (0.3 mg/mL in methanol) to turn it purple [8].
2.7. DPPH radical scavenging

We carried out DPPH antioxidant activity as described previously [9]. We dissolved DPPH radical in methanol at 0.025 mg/mL and left it at room temperature for 60 minutes. We used gallic acid, antioxidant as the reference. We prepared 700 μL of the methanolic solutions of LAD and LHM from a stock solution (10 mg/mL) at different concentrations (0, 1 μg at 100 μg/mL), then 1400 μL of DPPH solution was added. After 30 minutes of incubation in the dark, we measured the absorbance at 517 nm using a 6705 UV / Vis spectrophotometer JENWAY. We calculated percent inhibition according to the following equation:

\[
\% \text{ Inhibition} = \frac{A_b - A_s}{A_b} \times 100
\]

Where \(A_b\) is the absorbance of the blank and \(A_s\) is that of the sample. All samples were analyzed in triplicate and results were given as IC₅₀.

2.8. Acute toxicity assessment

To assess acute toxicity of LAD and LHM, we estimated the LD₅₀ of extracts following the Organization for Economic Development Cooperation (OECD) Guideline 423 [10].

2.9. Hepato-curative activity assessment

2.9.1. Preparation of doses of active substances

For experimental essays, we prepared LAD or LHM solution at 1:5, 1:10 and 1:100 of their respective estimated LD₅₀ of. Silymarin, a hepatoprotective substance used as reference, was administered at 100 mg/kg of body weight. The CCl₄ was diluted in olive oil (1:1 ratio) and used at 1 mL/kg of body weight.

2.10. Experimental design

We evaluated hepato-curative activity as previously described with slight modifications [11, 12]. After 12 hours of starvation, 45 male rats were randomized into nine (9) groups five (5) rats each. We administered intraperitoneally (ip) a single dose of 1 mL/kg body weight of diluted CCl₄ in olive oil (1:1 ratio) to induce acute CCl₄ intoxication and subsequent hepatitis. Rats accessed freely water one hour later, and food two hours after intoxication Treatment regimen are specified below.

- **Group 1**: Neutral controls: rats received olive oil (ip) on day 1 followed by distilled water (10 mL/kg per oral route) for seven (7) consecutive days.
- **Group 2**: Negative controls: rats were intoxicated with CCl₄ (ip) on day 1, followed by distilled water (10 mL/kg oral route) for seven (7) consecutive days.
- **Group 3**: Positive control: rats were intoxicated with CCl₄ (ip) day 1, then treated with silymarin (10 mg/kg per oral route) for seven (7) consecutive days.
- **Group 4-6**: Rats were intoxicated with CCl₄ (ip) day 1, then respectively treated with 400, 200 and 100 mg/kg per oral route of LAD from the mixture.
- **Group 7-9**: Rats were intoxicated with CCl₄ (ip) day 1, then respectively treated with 400, 200 and 100 mg/kg of LHM from the mixture.

2.11. Preparation of samples for biochemical and histological studies

Twenty-four (24) hours after the last administration, we weighed the rats and anesthetized them with ether. We drew whole blood from rats by cardiac puncture and stored the samples in dry sterile tubes. After coagulation, we centrifuged blood samples at 3000 RPM for 10 minutes to save serum. We used human diagnostic reagents kits in HumaStar 600™ (Catalogue number, country) to measure the levels of the following biomarkers: alanine amino transferase (ALT), aspartate amino transferase (AST), gamma-glutamyl transferase (γ-GT), alkaline phosphatase (ALP), total bilirubin (TB) and total protein (TP). After blood sampling, rats were sacrificed by beheading. Liver, spleen and kidneys were removed and weighed. We calculated relative weight of the organs in relation to the body weight using this formula [13].

\[
\text{Relative Weight} = \frac{\text{Liver weight}}{\text{Body weight}} \times 100
\]
The liver of each rat was fixed in 10% formalin aqueous solution for histopathological examination. We analyzed histologic sections of liver at 40X using the LEICA DM 750 Microscope with LAS EZ software (catalogue number, France).

2.12. Data analysis
We expressed our results as means ± SEM. We processed and analyzed data using Microsoft Office Excel 2015 software and GraphPad Prism® version 5.03, respectively. We compare multiple groups (neutral controls versus treated rats, negative controls versus treated rats, treated rats from different treatment regimen) using one-way ANOVA test followed by the Tukey test. Differences were considered statistically significant if p <0.05 (*), very significant if p <0.01 (**) and highly significant if p <0.001 (***)..

3. Results

3.1. Characterization of chemical groups
The reaction of characterization revealed the presence of several chemical groups mainly tannins, sugar, coumarins, leucoanthocyanins and mucilages, however we found no alkaloids, carotenoids and reducing compounds. We found coumarins, tannins, saponosides, flavonoids, sterols and triterpenes on the TLC plate.

3.2. Acute toxicity
Within four hours of a single dose of 2000 mg/kg of LAD and LHM from of combination, rats showed no behavioral changes. They remained mobile, sensitive to tactile and calm up to over the 14-day follow-up and no death was observed. The LD$_{50}$ was estimated to be greater than the 2000 mg/mL orally.

3.3. DPPH radical scavenging
Both lyophilizates from the crude extracts distinctly showed radical scavenging ability. After spraying with DPPH solution, the TLC plates showed yellow spots on a purple background lying on compounds migration lines. Yellow spots, indicating high radical scavenging activity, could be observed for either LAD or LHM. Quantitative determination of DPPH radical scavenging activities of the both lyophilizates revealed a very high antioxidant potency (Figure 1). The crude extracts LAD and LHM yielded IC$_{50}$ = 5.95 ± 0.01 µg/mL and IC$_{50}$ = 8.66 ± 0.02 µg/mL, respectively. In positive controls gallic acid and quercetin, IC$_{50}$ were 1.99 ± 0.02 and 2.09 ± 0.1 µg/mL, respectively.

![Figure 1 DPPH radical scavenging by LAD and LHM](image)

3.4. Relative weight of the organs
Relative organ weight did not show any significant difference (p > 0.05) between the different groups of rats (Table 1). The relative liver weight of the rats in the negative control group (3.82 ± 0.14 g) was greater than that of the other groups except the group which received 100 mg/kg of the LHM (3.83 ± 0.16 g). The relative weights of the spleen 0.26 ± 0.02 g and the kidneys 0.76 ± 0.07 g of the negative control group are greater than those of the other groups.
Table 1 Effect of daily administration for seven (7) consecutive days of LAD and LHM on the relative weight of liver, spleen and kidneys of the rats

<table>
<thead>
<tr>
<th>Groups (doses)</th>
<th>Relative organ weights (Mean ± Standard Error of the Mean)</th>
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<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Neutral control</td>
<td>3.63 ± 0.10</td>
</tr>
<tr>
<td>Negative control</td>
<td>3.82 ± 0.14</td>
</tr>
<tr>
<td>Positive control</td>
<td>3.66 ± 0.15</td>
</tr>
<tr>
<td>LAD 100 mg/kg</td>
<td>3.31 ± 0.24</td>
</tr>
<tr>
<td>LAD 200 mg/kg</td>
<td>3.60 ± 0.11</td>
</tr>
<tr>
<td>LAD 400 mg/kg</td>
<td>3.65 ± 0.13</td>
</tr>
<tr>
<td>LHM 100 mg/kg</td>
<td>3.83 ± 0.16</td>
</tr>
<tr>
<td>LHM 200 mg/kg</td>
<td>3.81 ± 0.12</td>
</tr>
<tr>
<td>LHM 400 mg/kg</td>
<td>3.71 ± 0.11</td>
</tr>
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</table>

The relative organ weights did not show a significant difference between the different groups of rats.

3.5. Effect of LAD and LHM on biochemical parameters

Carbon tetrachloride (CCl₄) resulted in a significant increase (p<0.001) in transaminases in untreated intoxicated rats (ALT = 82.64 ± 3.91 U/L; AST = 129.8 ± 4.02 U/L) compared to non-intoxicated rats (ALT = 43.36 ± 3.29 U/L; AST = 74.5 ± 3.12 U/L).

Figure 2 (a-b): Effect of daily administration for seven consecutive days of LAD, LHM and silymarin on transaminases. Values were expressed as mean ± SEM, n = 5. * p <0.05; ** p <0.01; *** p <0.001 significantly different as compared to negative controls; #p <0.05; ## p <0.01; ### p <0.001 significantly different as compared to neutral controls; + p <0.05; ++ p <0.01; +++ p <0.001 and - p <0.05; -- p <0.01; --- p <0.001 significant difference as compared to rats treated by other doses with 400 mg/kg dose of LAD and LHM respectively

The two lyophilizates at all doses (400, 200 and 100 mg/kg) caused a significant decrease of transaminases with ALT level respectively (LAD: 46.6 ± 2.5 U/L; 53.26 ± 1.79 U/L; 56.98 ± 2.25 U/L and LHM: 52.8 ± 1.35 U/L; 59.42 ± 2.3 U/L; 68.58 ± 4.1 U/L) and AST (LAD: 77.4 ± 3.07 U/L; 84.6 ± 2.65 U/L; 95.7 ± 4.28 U/L and LHM 85.8 ± 2.83 U/L; 89 ± 2, 93 U/L; 113.8 ± 3.33 U/L) compared to untreated intoxicated rats. The decrease of ALT level was only significant (p<0.001)
between 400 mg/kg of LAD compared to 100 mg/kg of LHM and (p<0.05) between 200 mg/kg of LAD and 400 mg/kg of LHM compared to 100 mg/kg of LHM. The decrease of AST level was significant (p<0.001) between 400, 200 mg/kg of LAD and LHM compared to dose 100 mg/kg of LHM. On average, rats treated with silymarin showed a significantly reduced ALT (45.76 ± 3.46 U/L) and AST (75.6 ± 3.72 U/L) levels as compared to rats treated with 100 mg/kg of LHM (p<0.001) as well a significantly reduced AST level as compared to rats treated with 100 mg/kg of LAD (p<0.01) (Figure 2: a-b). Lyophilizates at 400 mg/kg (LAD: 23.13 ± 5.61 U/L; LHM: 39.66 ± 2.52 U/L) and as well as silymarin (35.72 ± 12.81 U/L) decreased the level of γ-GT as compared to the untreated and intoxicated rats (93.6 ± 5.9 U/L) with a significant difference (p<0.001). Lyophilizates at 200 mg/kg (LAD: 41.63 ± 5.49 U/L; LHM: 52.83 ± 9.47 U/L) did not show any significant difference p<0.05. γ-GT level was only significantly reduced between 400 mg/kg of LAD as compared to 100 mg/kg of LHM (p<0.001), but no difference was observed with 200 mg/kg of LAD and 400 mg/kg of LHM as compared to 100 mg/kg of LHM (p<0.05).

Alkaline phosphatase (ALP) level in the untreated intoxicated rats (222.6 ± 9.69 U/L) was significantly reduced (p<0.05) by silymarin (168 ± 6.63 U/L) and 400 mg/kg of LHM (175.4 ± 9.46 U/L) and a greater reduction (p<0.01) by 400 mg/kg of LAD (156.6 ± 16.13 U/L). No significant difference was observed for the decrease in ALP levels between the different doses of LAD and LHM (Figure 3: a-b).

3.6. Effect of lyophilizates on the activity of other biochemical parameters

Untreated and intoxicated rats showed a significantly increased in the total serum bilirubin level (5.03 ± 0.43 µmol/L) as compared to non-intoxicated rats (2.13 ± 0.19 µmol/L) (p<0.01). After day 7 post-treatment with silymarin and 400 mg/kg of LHM, there was a significant reduction (p<0.01) in the level of total bilirubin with 2.54 ± 0.41 µmol/L and 2.38 ± 0.39 µmol/L, respectively. The 400 mg/kg doses of LAD and 200 mg/kg of LHM significantly (p<0.05) reduced the level of total bilirubin. The total serum protein level was significantly elevated (p<0.05) for rats treated with either silymarin (61.2 ± 0.68 g/L) or 400 mg/kg of LAD (61 ± 0.70 g/L) plus LHM (60.8 ± 0.96 g/L) as compared to untreated and intoxicated rats (56.4 ± 1.2 g/L). Total bilirubin and total protein levels showed no significant difference (P>0.05) between the different doses of LAD and LHM. (Figure 4: a-b).
3.7. Effect of lyophilizates on histopathological structure of the liver

Figure 4 (a-b): Effect of daily administration for seven consecutive days of LAD, LHM and silymarin on Total bilirubin and Total protein. Values were expressed as mean ± SEM, n = 5. * p <0.05; ** p <0.01; *** p <0.001 significantly different as compared to negative controls; #p <0.05; ## p <0.01; ### p <0.001 significantly different as compared to neutral controls; +p <0.05; ++ p <0.01; +++ p <0.001 and -p <0.05; -- p <0.01; --- p <0.001 significant difference as compared to rats treated by other doses with 400 mg/kg dose of LAD and LHM respectively.

Figure 5 (G1-9): Images of liver sections of rats at magnification 40 at the end of the experiments. G1: neutral control, G2: negative control, G3: positive control, G4: 400 mg/kg of LAD, G5: 200 mg/kg of LAD, G6: 100 mg/kg of LAD, G7: 400 mg/kg of LHM, G8: 200 mg/kg of LHM, G9: 100 mg/kg of LHM
The liver of the rats of group 1, the normal controls, exhibited a normal parenchyma (Fig.5: G1). Group 2 rats, those intoxicated with carbon tetrachloride and untreated, showed an unrecognizable trabecular liver organization, a centrilobular necrosis with congested and dilated centrilobular veins along a hepatocytic beach. (Fig.5: G2). All rats treated with silymarin, LAD and LHM showed a less or more improved liver architecture in a dose-dependent manner for lyophilizates. The sections of the liver from rats treated with silymarin 100 mg/kg showed rare punctuate peripheral necrosis in the lobules (Fig.5: G3). In rats treated with 400 mg/kg of LAD, rare hepatocytes showed cytoplasm’s that were slightly infiltrated with eosinophils (Fig.5: G4). Those treated with 200 mg/kg of LAD showed some hepatocyte clumps with no nucleus (Fig.5: G5). At 100 mg/kg B.wt. of LAD, the sections showed some isolated intralobular lymphocytic clusters (Fig.5: G6).

The LHM showed eosinophilic cytoplasm with lymphocytic clusters 400 mg/kg body weight (Fig.5: G7), diffuse dark nuclei with intraparenchymal lymphocytic clusters at 200 mg/kg body weight. (Fig.5: G8). A disseminated and diffuse lymphocyte with rare lymphocytic clusters was found in rats treated with 100 mg/kg body weight. (Fig.5 G9).

4. Discussion
Phytochemical screening revealed mainly the presence of tannins and coumarin. Saponosides, oses, sterols and triterpenes were present at a smaller amount. The LD50 of both extracts were estimated greater than 2000 mg/kg body weight by the oral route. So they were classified into category 5 toxicity, which the OECD Harmonized Classification System has considered non-toxic by oral route [10]. Non-toxicity of sole plant extract was reported in previous studies. Bayala et al, proved that *Leptadenia hastata* was nontoxic with a LD50 > 4000 mg/kg orally [14]. Haidara et al, found a LD50> 2000 mg/kg orally for *Terminalia macropera*. [15] *Cochlospermum tinctorium* and *Commiphora africana* were classified as practically non-toxic with a LD 50> 5000 mg/kg [16, 17]. The carbon tetrachloride induced-hepatitis has been used as an experimental model to study hepato-curative activity of natural compounds [11]. Ours results showed that AST, ALT, ALP and γ-GT in the CCl4 control group were almost twice higher as compared to the neutral control group (P <0.05) (Figure 2 a and 3). These results were similar to that of Arka et al.. [18]. In general, the return to normal level of transaminases corresponds to healing of the hepatic parenchyma and regeneration of hepatocytes [19]. The two lyophilizates initiate the processes of regeneration and repair of liver damage caused by CCl4. This could explain the reduction of the level of these enzymes in the blood as mentioned by other authors [20]. The two lyophilizates at all doses significantly reduced the level of transaminases (Figure 2: a-b). Except 100 mg/kg dose, 400 and 200 mg/kg doses of LAD significantly reduced alkaline phosphatase and gamma-GT as well as LHM at the 400 mg/kg dose (Figure 3: a-b). These results corroborate with those of Satyapal et al, who in his study tested a formulation based on seventeen plants not taking account our plants [21]. A significant reduction of total bilirubin was obtained with the 400 mg/kg dose of LAD and LHM as well as the 200 mg/kg dose of LHM as compared to the CCL4 group. Total protein assay showed a significant increase in the 400 mg LAD and LHM doses as compared to the negative controls (CCl4-treated) suggesting liver cell regeneration after the damage. The restoration of protein concentration is probably due to the increase in protein synthesis. Stimulation of protein synthesis was found to accelerate the regenerative mechanism of liver cells [22]. The hepato-curative activity of LAD and LHM could be explained by the presence of phytoconstituents in particular, polyphenols, coumarins, sterols and triterpenes, flavonoids and saponosides which are known to have radical scavenging capacity. Moreover, flavonoids, saponins, polyphenols, coumarins, triterpenes have been reported to have hepatoprotective effects [23]. Farghali et al, have shown that the hepatoprotective effect of silymarin was due to the inhibition of lipid peroxidation and the modulation of calcium ions in hepatocytes [24]. Liver enzymes enter the circulatory system after lesions of the hepatic parenchyma that alters membrane permeability [25]. This confirms the results of the histologic examination. The liver of the silymarin and the LAD and the LHM-treated rats intoxicated with carbon tetrachloride showed a more or less renewed to normal architecture of liver histology as compared to intoxicated and untreated rats with central-lobular necrosis with hepaticycanges. Thabrew et al, showed that the reduction in the blood of transaminases is linked to the repair of hepatic parenchyma and the regeneration of hepatocytes [19]. Both lyophilizates showed a dose-dependent decrease in AST/ALT, γ-GT and ALP levels, but LAD caused a greater decrease in these enzymes than LHM. Both lyophilizates showed a dose-dependent decrease in total bilirubin levels and a dose-dependent increase in total protein levels. LHM caused a greater decrease in total bilirubin levels than LAD and conversely a better increase in total protein levels. The chemical compounds revealed in both extracts were probably responsible for the hepatoprotective effect.

5. Conclusion
This study showed the hepato-curative activity of an extract based on combination of four medicinal plants *Commiphora Africana* (Burseraceae), *Cochlospermum tinctorium* (Bixaceae), *Leptadenia hastata* (Apocynaceae) and *Terminalia*
*macroptera (Combretaceae)*. After seven consecutive days of treatment with carbon tetrachloride; intoxicated rats showed at different doses of the lyophilizates from respective aqueous decoction (LAD) and aqueous methanolic maceration (LHM) a significant reduction in biomarkers. The histologic examination showed a recovery of liver damage towards normal architecture in dose-dependent manner for both lyophilizates. Our results confirm the hepato-curetive activity of the combination and justifies the validity of its traditional use for the treatment of hepatitis. In the future, we plan other studies to optimize the contribution of each individual plant and to understand mechanisms underlying its activity on the liver.

**Compliance with ethical standards**

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**Disclosure of conflict of interest**

The authors declare that they have no competing interests.

**Statement of ethical approval**

We strictly observed internationally accepted standards and principles for laboratory animal use. Our study protocol was approved by the Ethical Committee at the University Joseph Ki-ZERBO with a protocol approval # Reg. No. CE-UOI/2019-07.

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