Hepatoprotective Activity of Aqueous Acetone Extract from *Cienfuegosia digitata* Cav. (Malvaceae) Against Alcohol Hepatotoxicity in Albinos Wistar Rats

1K. Konaté, 2M. Ouédraogo, 1M.B. Ouattara, 1I.C. Dibala, 4J. F. Mavoungou, 5A.N. Lepengué, 6A. Souza, 5B.M. Batchi and 1O.G. Nacoulma

1Laboratoire de Biochimie et Chimie Appliquées (LABIOCA), UFR/SVT, Université de Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso
2Laboratoire de Physiologie Animale, UFR/SVT, Université de Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso
3Laboratoire de Biochimie Alimentaire, Enzymologie, Biotechnologie et Bioinformatique, UFR/SVT, Université de Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso
4Institut de Recherche en Ecologie Tropicale (IRET/CENAREST). BP: 13354 Libreville-Gabon
5Laboratoire de Phytopathologie, UFR Agrobiologie, Faculté des Sciences, Université des Sciences et Techniques de Masuku, BP 943 Franceville-Gabon
6Laboratoire de Physiologie Animale, Electrophysiologie et de Pharmacologie, Faculté des Sciences, Université des Sciences et Techniques de Masuku, Franceville-Gabon

Abstract: The present study was conducted to evaluate the hepatoprotective effects of aqueous acetone extract of *Cienfuegosia digitata* Cav. (Malvaceae) an herbal plant used in Burkina Faso to treat traditionally liver disease in albinos Wistar rats. Animals received by gavage 25, 50 and 100 mg/kg body weight of extract daily for a period of 28 days. Control groups received alcohol 35% and water. In vivo administration of 35% ethanol for a period of 28 days in rats showed an activity of liver marker enzymes (AST, ALT, ALP and GGT), triglycerides, total cholesterol, total bilirubin and direct bilirubin in serum compared with rats which received water (control water). However, administration of 35% ethanol along with aqueous acetone extract decreased the activities of liver markers enzyme in serum comparatively to the control water group (p<0.05 or p<0.01). We noticed that extract at a dose of 100 mg/kg body weight was highly effective than 25 and 50 mg/kg body weight compared to the control water group (ALT and ALP; p>0.05 and p<0.05). This study revealed that *Cienfuegosia digitata* presents a hepatoprotective potential and this plant could be traditionally exploited in the treatment of liver disease particularly hepatitis B in Burkina Faso.

Key words: *Cienfuegosia digitata* Cav, biochemical parameters, hepatoprotective activity, Albinos Wistar rats, alcohol, Hepatotoxicity

INTRODUCTION

The liver is the largest and most complex organ in the human body and has a unique and remarkable power to regenerate. It carries out a wide range of exocrine and endocrine functions such as maintenance of carbohydrate homeostasis, storage of nutrients, secretory and excretory functions, protein synthesis and a couple of vital metabolic processes including hormone, lipid and protein metabolism and detoxification of xenobiotics mediated by cytochrome P450-dependent oxidases and conjugates (Guyton *et al*., 1981). The wide range of its functions makes the liver vulnerable to a variety of disorders which can be broadly classified into acute or chronic hepatitis (inflammatory disease), hepatosis (non-inflammatory disease) and liver cirrhosis (Wagner *et al*., 1980). Although viruses are the leading cause of liver diseases, liver lesions arising from therapeutic agents and alcohol (Passmore and Robinson, 1973). Thus liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health. The drugs available in the modern system of medicine are corticosteroids and which bring about symptomatic relief without cure and their use is associated with the risk of relapses and dangerous side effects. Lack of therapeutic agents in modern medicine and the use of a vast array of plants in traditional practices warrant investigation of these plants using modern techniques to validate hepatoprotective claims and to search for novel molecules for use against liver toxicity. A review of the literature...
shows that plant will prove to be the most fertile ground to look for useful liver drugs (Handa et al., 1986). Among them we have Cienfuegosia digitata Cav.

Cienfuegosia digitata Cav., Malvaceae species, is found in several parts of the country and is very widely used in traditional medicine in Burkina Faso. The aqueous extract was used against hepatitis B virus cure in the central region of Burkina Faso and various kinds of diseases such as malaria, hepatic disorders and alcoholic hepatitis (Nacoulma, 1996). Phytochemical analysis of this plant demonstrated the presence of saponosides, coumarins, steroids, polyphenols/tannins, flavonoids and alkaloids (Konaté and Souza, 2010).

Considering the potential claims of this Malvaceae species and because the plant has hepatoprotective properties, the objective of this present study was undertaken to evaluate the hepatoprotective activity against alcohol hepatotoxicity in albinos Wistar rats in order to provide a scientific basis for the traditional use of Cienfuegosia digitata for treating hepatitis B in Burkina Faso.

MATERIALS AND METHODS

Plants material: Cienfuegosia digitata Cav. was collected in August 2008 in Gampela, 25 km east of Ouagadougou, capital of Burkina Faso. The plant was botanically identified by Prof. Millogo-Rasolodimby from the plants Biology Department of the University of Ouagadougou. A voucher specimen was deposited at the Herbarium of the Laboratoire de Biologie et d’Ecologie Végétale, UFR/VT of University of Ouagadougou.

Animals: We used male and female adult albinos Wistar rats (200-210 g) coming from University of Yaoundé (Cameroon). The animals were housed in cage under controlled conditions of 12-h light/12-h dark cycle and 25°C. They all receive pellets food enriched with protein and water ad libitum. The animals were fed with pellets of the cattle food of Libreville (Gabon), containing 20% of proteins and water.

Experimental design for hepatoprotection potential: The rats were divided randomly into five groups of six rats each. The first and second groups served as control, received water and 35% ethanol. The remaining groups (group 3; group 4; group 5) received three dose levels of aqueous acetone extract of Cienfuegosia digitata (25, 50 and 100 mg/kg bw) suspended in 35% of ethanol, administered orally by gavage daily for a period of 28 days. Body weight was measured weekly and the animals were observed daily for signs of abnormalities throughout the study. At the end of a period of 28 days, the animals were deprived of food for 15 h. Blood samples were collected by cardiac puncture for biochemical.

Assessment of hepatoprotective potential: Blood samples were collected by cardiac puncture in three tubes for haematology, glucose and serum biochemistry. The blood samples with heparin and without anticoagulant were centrifuged at 3000 rpm for 5 min to obtain plasma or serum. Plasma was used to determine glucose (Burn and Price, 1985; Trinder, 1969) and the serum for other biochemical parameters such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) determined according to Schumann et al. (2002a) and Schumann et al. (2002b), alkaline phosphatase (ALP) was estimated by Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974) and German Society for Clinical Chemistry (1972) gamma glutamyl transferase (GGT) was determined according to Schumann et al. (2002a) total bilirubin and direct bilirubin were determined according to Sherwin and Thompson (2003), triglycerides (Fossati and Prencipe, 1982), total cholesterol (Allain et al., 1974). All these biochemical parameters were measured by Selectra XL Vital Scientific (Elitech Group Company).

Statistical analysis: The data were expressed as Mean±Standard Deviation (SD) of six determinations (n = 6). Results were analyzed by one-way ANOVA followed by Dunnett’s t-test using Prism 4 software. The level of significance was accepted at p ≤ 0.05.

RESULTS

Effects of extract on body weights in rats: The results consigned in Table 1 are related to the body weights and the treatment period. Statistical studies revealed that there is a low significant difference in body weight gain between control groups and the treated assay groups during the three weeks (p<0.05 or p<0.01). The results underline also an increase of animal body weights as a function of treatment period. However, in the fourth week, there was a significant difference in body weight gain between the test groups and the control alcohol group (p<0.01).

Effects of extract on organs weights in rats: Table 2 shows the effects of aqueous acetone extract of Cienfuegosia digitata Cav. on the weights of vital organs of rats. It is found that the effect of aqueous acetone extract of Cienfuegosia digitata Cav. to the concentration of 25 and 50 mg/kg bw concentrations causes a significant decrease of liver weight (p<0.05) and heart (p<0.01 and p<0.05) compared to the control group (10% DMSO). However, there is no significant difference between the effect of the aqueous acetone extract of Cienfuegosia digitata Cav. at different concentrations on the variation of the weights of other organs and the control groups (p>0.01).

Assessment of hepatoprotective potential: Table 3 shows the effects of aqueous acetone extract of Cienfuegosia digitata Cav. extract on the biochemical parameters. AST (25; 50 and 100 mg/kg; p<0.01),
Table 1: Animal weights (g) with time of treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>First day</th>
<th>First week</th>
<th>Second week</th>
<th>Third week</th>
<th>Fourth week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>207.33±12.69</td>
<td>223.5±3.83</td>
<td>235.00±10.95</td>
<td>243.5±14.79</td>
<td>253.00±19.72</td>
</tr>
<tr>
<td>Group 2</td>
<td>207.00±13.18</td>
<td>221.00±6.57</td>
<td>205.00±5.48</td>
<td>189.33±13.69</td>
<td>183.00±3.29</td>
</tr>
<tr>
<td>Group 3</td>
<td>206.5±13.07</td>
<td>213.00±9.86*</td>
<td>201.00±10.95*</td>
<td>178.00±14.24**</td>
<td>186.00±1.10**+</td>
</tr>
<tr>
<td>Group 4</td>
<td>207.00±3.18</td>
<td>218.00±1.64</td>
<td>215.67±30.94</td>
<td>190.00±16.43**</td>
<td>192.33±1.37**</td>
</tr>
<tr>
<td>Group 5</td>
<td>207.17±13.91</td>
<td>217.00±5.54</td>
<td>213.00±20.81</td>
<td>197.00±13.69**</td>
<td>200.5±10.41**+</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M. (n = 6) one-way ANOVA followed by Dunnett’s t-test: Compare all vs. control; *: p<0.05; **: p<0.01 compared with control water; +: p<0.05 with control alcohol

Group 1: control water, rats received water
Group 2: control alcohol, rats received 35% ethanol
Group 3: rats received 35% ethanol with extract (25 mg/kg body weight)
Group 4: rats received 35% ethanol with extract (50 mg/kg body weight)
Group 5: rats received 35% ethanol with extract (100 mg/kg body weight)

Table 2: Effects of aqueous acetone extract of *Cienfuegosia digitata* on organs weights (g) of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Left kidney</th>
<th>Right kidney</th>
<th>Stomach</th>
<th>Lungs</th>
<th>Liver</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0.73±0.02</td>
<td>0.75±0.11</td>
<td>3.33±0.55</td>
<td>1.78±0.016</td>
<td>7.609±0.055</td>
<td>0.804±0.00</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.68±0.001</td>
<td>0.63±0.05</td>
<td>3.02±0.00</td>
<td>1.73±0.219</td>
<td>4.610±0.002</td>
<td>0.63±0.19</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.65±0.01*</td>
<td>0.64±0.55*</td>
<td>3.17±0.00*</td>
<td>1.74±0.18**</td>
<td>5.38±0.00**+</td>
<td>0.65±0.03*</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.67±0.0*</td>
<td>0.69±2.19*</td>
<td>3.19±1.64 ns</td>
<td>1.76±0.02ns</td>
<td>5.38±0.00**++</td>
<td>0.69±0.2**</td>
</tr>
<tr>
<td>Group 5</td>
<td>0.69±0.00*</td>
<td>0.71±0.0+</td>
<td>3.22±1.10*</td>
<td>1.76±0.013ns</td>
<td>5.80±0.03**++</td>
<td>0.76±0.3*+</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M. (n = 6) one-way ANOVA followed by Dunnett’s t-test: Compare all vs. control; *: p<0.05; **: p<0.01 compared with control water; +: p<0.05 with control alcohol

Group 1: control water, rats received water
Group 2: control alcohol, rats received 35% ethanol
Group 3: rats received 35% ethanol with extract (25 mg/kg body weight)
Group 4: rats received 35% ethanol with extract (50 mg/kg body weight)
Group 5: rats received 35% ethanol with extract (100 mg/kg body weight)

Table 3: Effects of aqueous acetone extract of *Cienfuegosia digitata* on the biochemical parameters in the plasma and the serum of rats

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (UI/l)</td>
<td>87.5±0.82</td>
<td>154.75±4.40</td>
<td>141.83±9.06**++</td>
<td>123.58±6.17**++</td>
<td>101.85±3.94**++</td>
</tr>
<tr>
<td>ALT (UI/l)</td>
<td>31.5±7.12</td>
<td>58.5±1.64</td>
<td>49.00±2.19**++</td>
<td>51.5±1.64**+</td>
<td>38.13±0.03**++</td>
</tr>
<tr>
<td>ALP (ALP/l)</td>
<td>76.63±3.97</td>
<td>138.5±20.27</td>
<td>111.00±17.53**</td>
<td>109.04±39.62*</td>
<td>83.88±3.97++</td>
</tr>
<tr>
<td>GGT (UI/l)</td>
<td>6.73±0.52</td>
<td>14.23±1.71</td>
<td>10.93±1.02**++</td>
<td>10.04±0.07**++</td>
<td>8.47±0.26**++</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.95±0.06</td>
<td>3.24±0.03</td>
<td>1.85±0.05**++</td>
<td>1.22±0.05*****</td>
<td>1.11±0.03**++</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>2.33±0.03</td>
<td>5.68±0.03</td>
<td>3.29±0.02**++</td>
<td>2.73±0.03*****</td>
<td>2.41±0.05**++</td>
</tr>
<tr>
<td>Total bilirubin (mmol/l)</td>
<td>0.08±0.007</td>
<td>0.187±0.007</td>
<td>0.170±0.000**</td>
<td>0.140±0.002**++</td>
<td>0.099±0.034++</td>
</tr>
<tr>
<td>Direct bilirubin (mmol/l)</td>
<td>0.014±0.000</td>
<td>0.047±0.001</td>
<td>0.041±0.0001**</td>
<td>0.028±0.005**++</td>
<td>0.019±0.003**++</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>7.5±0.11</td>
<td>10.1±0.22</td>
<td>9.85±0.05**++</td>
<td>9.5±0.11**++</td>
<td>7.75±0.16**++</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M. (n=6) one-way ANOVA followed by Dunnett’s t-test: Compare all vs. control; *: p<0.05; **: p<0.01 compared with control Water; +: p<0.05; ++: p<0.01 compared with control alcohol

Group 1: control water, rats received water
Group 2: control alcohol, rats received 35% ethanol
Group 3: rats received 35% ethanol with extract (25 mg/kg body weight)
Group 4: rats received 35% ethanol with extract (50 mg/kg body weight)
Group 5: rats received 35% ethanol with extract (100 mg/kg body weight)

ALT (25; 50 and 100 mg/kg; p<0.01 and p<0.05), ALP (25; 50 and 100 mg/kg; p<0.01 and p<0.05), GGT (25; 50 and 100 mg/kg; p<0.01), total cholesterol (25; 50 and 100 mg/kg; p<0.01), total bilirubin (25; 50 and 100 mg/kg; p<0.01), direct bilirubin (25; 50 and 100 mg/kg; p<0.01 and p<0.05) and glucose (25; 50 and 100 mg/kg; p<0.01 or p<0.05) were significantly changed in the treated assay groups compared to the control groups (Water and 35% ethanol).

DISCUSSION

About the effect of the extract on the body weights of rats the results consigned in Table 1 showed that there is a relationship between the effect of the extract, the body weight of rats and the duration of the treatment. As a result, we noted a decrease in the body weights of rats. In addition, some studies reported that body weight is a simple and sensitive index of toxicity after exposure to toxic substance (Konaté et al., 2011). There is a close relation between alcohol exposition and the development of oxidative stress. Alcohol not only enhances ROS generation, but also depletes antioxidants, thus, creating a state of oxidative stress (Tuma and Casey, 2003). In addition, much of the direct cell injury that occurs during alcohol consumption is believed to be caused by accumulation of acetaldehyde, a toxic by-product of alcohol metabolism (Tuma and Casey, 2003). Therefore the overall biochemical implications of the deleterious effects of the toxic acetaldehyde and ROS on the liver are, in part, the composite signs of liver affection. The decrease of the body weights of rats is direct result of liver affection signs caused by alcohol. Comparatively to the control group (water), this decrease of the body weight...
weights of rats is low (100 mg/kg bw). As a result, we could say that Cienfuegosia digitata extract (100 mg/kg bw) possess a hepatoprotective potential. Our results consigned in Table 1 showed this report.

AST and ALT are the reliable makers for liver function. It is established that AST can be found in liver, cardiac muscle, skeletal muscle, kidney, brain, pancreas, lungs, leukocytes and erythrocytes where that ALT presence in liver (Rej et al., 1978). The increased levels of serum enzyme such as AST and ALT indicate the increased permeability and damage and/or necrosis of hepatocytes (Goldberg and Watts, 1965). The membrane bound enzymes like ALP and GGT are released unequally in to bloodstream depending on the pathological phenomenon (Sillanaukee, 1996). In our study, we have found that ethanol consumption caused a significant increased in the activities of AST, ALT, ALP and GGT, which could be to severe damage to tissue membrane. But these decreased activities of these enzymes indicate the hepatoprotective potential of our extract (100 mg/kg). This phenomenon could be explained by the presence of biologically active antioxidants found in Cienfuegosia digitata (Nacoulma, 1996) sparing antioxidant activity and reduced the consumption of endogenous antioxidants, which could be responsible for the reduction of oxidative stress during ethanol toxicity. The active constituents such as flavonols, flavonol oligomers and proanthrocyanidins were found in Cienfuegosia digitata (Nacoulma, 1996) and it has been reported as powerful antioxidants (Fauconneau et al., 1997). Phytochemical analysis carried out on this pant extract showed that Cienfuegosia digitata contains highly in polyphenolic compounds (Nacoulma, 1996). Thus, the restoration of antioxidant defense system in this study may be attributable in part, to the antioxidant sparing action of the polyphenolic compounds (Sane et al., 1995; Calixo et al., 1998). Moreover, polyphenols could be probably responsible for the hepatoprotective potential of Cienfuegosia digitata. Moreover, our results revealed a direct correlation between liver weights decreased significantly compared to the controls group (Water and 35% ethanol), the reliable makers for liver function (AST, ALT and GGT), ALP, glucose, triglycerides, total cholesterol, total bilirubin and direct bilirubin significantly increase or decrease (p<0.01 or p<0.05) compared to the control groups (water and 35% alcohol). Indeed, these reports could be explained clinically. It is well known that medically an increase in cholesterol levels also cause increase in triglycerides enzymes sensitive to cytotoxic injury are serum Alamine amino Transferase (ALT) and serum Aspartate amino Transferase (AST). ALT is a sensitive indicator to acute liver damage and elevation of this enzyme in no hepatic disease is unusual. ALP, although is not a liver specific enzyme and the liver is major source of this enzyme. Also the levels of this enzyme increase in cholestasis, elevated serum GGT levels appear to be indicative of diseases of the liver, biliary tract and pancreases. Bilirubin levels in blood also increase in liver diseases. The increased of the reliable makers for liver function results also the increased of glucose in the plasma. In short we noticed an increase of biochemical parameters comparatively to the control alcohol group. But the decreased activities of these same parameters indicate the hepatooprotective potential of our extract (100 mg/kg bw).

CONCLUSION

At the end of our study it appears clear that aqueous acetone extract of Cienfuegosia digitata has a potential hepatoprotective. However, we do not notice any dose-response. As a result, this Malvaceae could be use in the treatment of hepatitis B in Burkina Faso.

ACKNOWLEDGMENT

The authors are grateful to the France Embassy in Burkina Faso/EGIDE-France for the mobility scholarship. The authors think also Prof. Millogo Rasolodimby from the plants Biology Department of the University of Ouagadougou for the botanically identified of plants.

REFERENCES


