INTRODUCTION

Herbal remedies are widely used for prevention and treatment of various diseases in Africa and developing countries (Islam et al., 2007). They are sources of natural substances used in treatment of many diseases (Submarawa et al., 2007). *Gomphrena celosioides* is an Amaranthaceae. Over 140 species of the same family are in America, including 46 in Brazil. Very few species are present in East and West Africa (Vieira et al., 1994). This weed of lawns, vacant lots and fields, was probably introduced in West Africa where it is now widespread. If in South America, it is used as abortives (Burkill, 1984), in Nigeria it is used for the treatment of dermatological (Onocha et al. 2005). In Benin, traditional healers make use of this plant in the treatment of many diseases including liver diseases, malaria and dysmenorrhea (Adjanohoun et al. 1989). Gesslers (1994), Vieira (1994) have demonstrated the analgesic, tonic, carminative and diuretic properties of this plant. Recently, Dosumo et al. (2010), reported its antimicrobial and anti hemlinic properties. The work of Botha et al. (1986) revealed the presence of saponins, steroids, amino acids, non-reducing sugars, phenols and flavonoids in this plant. These results were confirmed by de Moura et al. (2004). However, little information exists on hepatoprotective properties of this plant. It is therefore important works be undertaken to provide a scientific basis for using this plant in the treatment of liver diseases. Rauen and Schriewer (1971) have shown that silymarin administered orally opposes the increase in serum transaminases due to poisoning by tetrachloride carbon. We therefore considered interesting to investigate the effects of this hepatoprotective plant in comparison with that of silymarin. This study is conducted on Wistar rats whose livers are intoxicated with tetrachloride carbon (CCl4).

MATERIALS AND METHODS

**Materials**

Animal material consists of 63 Wistar rats of both sexes, aged of 8 months, average weight equal to 260 ± 20 g, obtained at the International Centre for Research-Development of Animal Husbandry in sub-humid areas of Bobo-Dioulasso (Burkina-
Faso). These animals are housed in conditions and environmental standards, fed a standard diet of rodents, water ad libitum, with care and treatment conditions, consistent with the guidelines of the Organization for Economic Cooperation and Development (OECD, 2008). Plant material consists of freeze-dried stems with leaves of *Gomphrena celosioides* harvested at NDali, North East of Benin with 500 km from Cotonou in October 2011. The botanical identification of the species was made by taxonomists of the National Herbarium of the University of Abomey - Calavi (UAC) in Benin. Sample documents have been filed in the same Herbarium. The identification was made under the number 6335/HNB AA. Tetrachloride Carbon, provided by UBC.HR. 6172 Leuven Belgium, is used for induction of liver toxicity. The extra virgin olive oil brand Belle France (Francap, BP 30403-75564 Paris Cedex 12) is used for preparation of intoxication. The Legalon®, Lot B 0902953, manufactured by MADAUS GmbH 51101 Cologne Germany is used as reference product. It contains 70 mg of silymarin. An analytical balance Sartorius type was used to weigh animals and their organs. Manipulations occured in Laboratory of Animal Physiology and Pharmacology at the Faculty of Science and Technology, University of Abomey-Calavi (Benin).

### MATERIALS AND METHODS

Experimentations were performed on nine batches of seven rats. Five batches served as controls and four experimentations with actual tests including preventive and curative tests. All rats were weighed at the beginning of test. The solutions of EAG, SIL, and intoxication are prepared before each test. The different treatments are done daily and at the same time. The animals were fasted for 12 hours and water only one hour before handling. They are fed an hour after the manipulations. Twenty-four hours after the last treatment, animals were weighed and anesthetia with ether. Their blood was collected by cardiac puncture into dry tubes and serum was used to estimate levels of serum transaminases: aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB) and conjugated bilirubin in plasma and serum. The extra virgin olive oil brand Belle France (Francap, BP 30403-75564 Paris Cedex 12) is used for preparation of intoxication. The Legalon®, Lot B 0902953, manufactured by MADAUS GmbH 51101 Cologne Germany is used as reference product. It contains 70 mg of silymarin. An analytical balance Sartorius type was used to weigh animals and their organs. Manipulations occurred in Laboratory of Animal Physiology and Pharmacology at the Faculty of Science and Technology, University of Abomey-Calavi (Benin).

#### Assays were carried out according to the IFCC enzyme kinetics. The principle is the determination of activity of GOT or GPT according to the following reactions:

**GOT:**

\[
\text{L-aspartate + 2-Oxoglutarate} \rightarrow \text{oxaloacetate + L-glutamate oxaloacetate + NADH + H}^+ \rightarrow \text{NAD}^+ + \text{L-malate}
\]

**ALT:**

\[
\text{alanine + alpha-ceto pyruvic acid glutamate + glutamate} \rightarrow \text{Pyruvic acid + NADH + H}^+ \rightarrow \text{NAD}^+ + \text{lactate}
\]

The decrease in absorbance due to conversion of NADH to NAD⁺ and proportional to the activity of GOT (or GPT) is measured at 340 nm.

### Bilirubin dosage

This test allows the colorimetric determination of total and conjugated bilirubin in plasma and serum. The determination of total bilirubin (TB) is performed in presence of dimethyl sulfoxide (DMSO) as a diazotization reaction with diazotized sulfanilic acid.

\[
\text{Sulfanilic acid + NaNO}_2 \rightarrow \text{Diazonium}
\]

\[
\text{DMSO dissolved in the aqueous phase unconjugated bilirubin.} \quad \text{Diazonium}
\]

\[
\text{Presence of hydrochloric acid prevents the diazotization of}
\]

\[
\text{unconjugated bilirubin in the assay without DMSO.}
\]

In both cases, the intensity of the color of the diazo compound formed is proportional to the amount of bilirubin present in the sample.

### Controls

To verify the effects of different substances used for experiments on animals, batches 1, 2, 3, 4, 5, (control groups), are respectively given: water (H₂O) per os, 0,5 ml Olive oil (HO) intraperitoneally (IP) for 4 days, 0,5 ml of Tetrachloride carbon (CCl₄) per kg BW, by IP for 4 days (Kamssouloum, 1984); 500mg/kg PV of the aqueous extract of *Gomphrena celosioides* (EAG) orally for 5 days; PV 300mg/kg of silymarin (SIL) orally for 5 days.

### Preventive treatment

Preventive therapy (PT) highlights the preventive properties of EAG in comparison with that of SIL on batches 6 and 7. The rats of batch 6 each receive 500 mg / kg BW of EAG orally for 5 days followed by 0.5 ml / kg BW of CCl₄ in IP for 4 days. The rats of batch 7 receive 300 mg / kg BW of SIL orally for 5 days, then 0.5 ml / kg BW of CCl₄ in IP for 4 days.

### Cure

The cure (TC) highlights the healing properties of EAG in comparison with that of SIL on batches 8 and 9. The rats of batch 8 receive IP, 0.5 ml / kg BW per day of CCl₄ for 4 days then 500 mg / kg BW of the EAG orally for 5 days. The rats of batch 9 receive 0.5 ml / kg BW of CCl₄ per day for 4 days then 300 mg / kg BW of SIL orally for 5 days.

### Processing and data analyses

Data entry is performed using Excel 2007.

- Calculation of relative weights (RW)
  
  \[ \text{RW} = \text{Liver Weight/ Body weight x 100} \]

- Calculation of the percentage of protection (Performance)
  
  \[ \% \text{ protection} = \text{Control datas – after treatments datas/ Control datas} \]
Significance tests of treatments were performed by the GLM procedure of SAS 9.1 (SAS Institute, Inc., Cary, NC, USA). Comparisons of mean levels of significant factors were performed by Student Newman Keuls method.

RESULTS

The results obtained are summarized in Tables 1 and 2, the graph 1 and Figures 1,2,3,4,5.

Table 1: Treatment effects on change in body weight and liver weight in Wistar rats and comparison of means ± standard deviations (by level of treatment)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>d_weight</th>
<th>P_liver</th>
<th>P_rel</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>4.29 ± 1.80</td>
<td>13.71 ± 1.80</td>
<td>5.63 ± 0.71</td>
</tr>
<tr>
<td>HO</td>
<td>4.86 ± 0.38</td>
<td>14.86 ± 1.07</td>
<td>6.11 ± 0.44</td>
</tr>
<tr>
<td>CCl4</td>
<td>12.00 ± 0.58</td>
<td>10.00 ± 1.63</td>
<td>4.24 ± 0.66</td>
</tr>
<tr>
<td>SIL</td>
<td>8.00 ± 1.15</td>
<td>13.71 ± 1.80</td>
<td>5.70 ± 0.74</td>
</tr>
<tr>
<td>EAG</td>
<td>8.14 ± 0.70</td>
<td>13.71 ± 1.80</td>
<td>5.70 ± 0.73</td>
</tr>
<tr>
<td>SIL_CCl4</td>
<td>8.29 ± 0.76</td>
<td>14.29 ± 1.80</td>
<td>5.99 ± 0.75</td>
</tr>
<tr>
<td>EAG_CCl4</td>
<td>8.57 ± 0.98</td>
<td>13.42 ± 1.90</td>
<td>5.68 ± 0.80</td>
</tr>
<tr>
<td>CCl4_SIL</td>
<td>9.71 ± 0.76</td>
<td>12.57 ± 1.51</td>
<td>5.34 ± 0.64</td>
</tr>
<tr>
<td>CCl4_EAG</td>
<td>9.86 ± 0.38</td>
<td>12.00 ± 1.63</td>
<td>5.06 ± 0.69</td>
</tr>
</tbody>
</table>

*** = p <0.001; In the same column, treatment means hit with the same letters are not significantly different. d_weight is the average weight changes, p_liver is liver weight end of the experiment and p_rel is the relative liver weight.

Table 2: Treatment effects on transaminases, alkaline phosphatase and bilirubin in Wistar rats and comparison of means (by level of treatment)

<table>
<thead>
<tr>
<th>Traitement</th>
<th>ASAT</th>
<th>ALAT</th>
<th>PAL</th>
<th>BT</th>
<th>BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>38.43 ± 1.51</td>
<td>41.14 ± 2.19</td>
<td>80.00 ± 27.54</td>
<td>6.00 ± 1.73</td>
<td>2.00 ± 0.82</td>
</tr>
<tr>
<td>HO</td>
<td>37.86 ± 4.10</td>
<td>41.57 ± 3.26</td>
<td>78.43 ± 27.45</td>
<td>5.00 ± 1.41</td>
<td>1.00 ± 0.82</td>
</tr>
<tr>
<td>CCl4</td>
<td>200.71 ± 13.66</td>
<td>172.14 ± 13.83</td>
<td>148.57 ± 22.55</td>
<td>18.00 ± 5.07</td>
<td>6.00 ± 1.73</td>
</tr>
<tr>
<td>SIL</td>
<td>45.00 ± 1.73</td>
<td>50.86 ± 1.57</td>
<td>75.00 ± 1.63</td>
<td>6.00 ± 1.29</td>
<td>2.00 ± 1.29</td>
</tr>
<tr>
<td>EAG</td>
<td>38.42 ± 2.37</td>
<td>39.86 ± 2.60</td>
<td>75.00 ± 19.10</td>
<td>6.00 ± 1.15</td>
<td>2.00 ± 0.82</td>
</tr>
<tr>
<td>SIL_CCl4</td>
<td>35.00 ± 1.15</td>
<td>37.29 ± 1.80</td>
<td>70.43 ± 15.54</td>
<td>6.00 ± 0.82</td>
<td>2.00 ± 1.00</td>
</tr>
<tr>
<td>EAG_CCl4</td>
<td>40.43 ± 2.37</td>
<td>60.14 ± 1.77</td>
<td>76.57 ± 18.61</td>
<td>5.71 ± 1.38</td>
<td>2.14 ± 0.90</td>
</tr>
<tr>
<td>CCl4_SIL</td>
<td>45.00 ± 1.73</td>
<td>50.86 ± 1.57</td>
<td>75.00 ± 1.63</td>
<td>6.00 ± 1.29</td>
<td>2.00 ± 1.29</td>
</tr>
<tr>
<td>CCl4_EAG</td>
<td>55.29 ± 1.11</td>
<td>75.14 ± 2.60</td>
<td>80.71 ± 1.80</td>
<td>10.00 ± 2.16</td>
<td>4.00 ± 216</td>
</tr>
</tbody>
</table>

*** = p <0.001; In the same column, treatment means hit with the same letters are not significantly different. AST and ALT are transaminases, alkaline phosphatase is PAL, BT’s total bilirubin, conjugated bilirubin is BC.

Graph 1: Comparative yields of liver enzymes

Table 2 shows the effects of different treatments on transaminase levels of alkaline phosphatase and bilirubin and the comparison of means (by level of treatment). Graph 1 present compared yields of liver enzymes. Results obtained with control animals that received only H2O and those who received only HO are in compliance with standards which are:

Biochemical parameters
AST 0-40 IU / l; ALT from 10 to 45 IU / l; PAL 30 to 125 mg / l; BT from 03 to 10 mg / l; BC from 01 to 03 mg / l. Against administration of the EAG of 200.71 ± 1.73 IU / l to 55.29 ± 1.11 IU / l for AST and 172.14 ± 13.83 IU / L to 75.14 ± 2.60 IU / l for ALT guards with respective 72.46% and 56.35% against 77.58% for EAG and 70.45% for SIL. All test results protectors (TP) point out that with the administration of the EAG there is a hepatoprotective activity significantly comparable to that of SIL with a protection of 79.86% to 85.56% against the EAG for SIL regarding AST and 65.06% to 78.34% against the EAG to SIL in respect of ALT. The results of alkaline phosphatase (ALP) and bilirubin (BT and BC) are significant (p <0.001) and in accordance with the standards for all treatments except with CCl4. Their percentages of protection are higher 45.67 ± 1.80% for PAL and 44.44 ± greater than 2.16% for BT.

**Histological parameters**

The results of histological studies are grouped pictures of microscopic sections of liver.

Figures 1-5 show the histological sections of liver of different groups of experimental animals, observed at 40X. The liver of batch 1 rats, normal control, shows a normal lobular architecture, marked by the presence of hepatocellular spans radiairement arranged around a central vein (CV). These bays are separated by sinusoids. In animals treated with the HO batch 2, batch 4 treated with EAG and batch 5 treated with SIL, the hepatic architecture is generally preserved. In animals of batch 3, poisoned with CCl4, the trabecular organization of the liver is unrecognizable (Figure 1). There is a massive hepatocyte necrosis with centriflobular vacular degeneration, a caryopyenose, a karyolysis and cytoplasmic acidophilia predominantly périlobulaire. That hepatocyte necrosis is accompanied by congestion of sinusoids and centriflobular veins dilated.

After administering a preventive treatment to the EAG for 5 days, followed by CCl4 intoxication (lot 6), liver lesions are less marked: the hepatic architecture remains recognizable but there are a few hepatocytes in the periphery of lobules with signs of necrosis including acidophilia cytoplasm and pyknosis of nuclei. Around the centrlobular veins are nearly normal hepatocytes (Figure 2). Rats of Lot 7 having received preventive treatment with SIL for 5 days followed by CCl4 intoxication (Figure 3) liver lesions observed are faint and are just a few hepatocytes acidophilia on perilobular region. After the cure for the EAG animals poisoned by CCl4 (lot 8), hepatocyte necrosis observed is less important than in animals intoxicated and untreated. The liver is generally recognizable

by the results are very high in animals only CCl4. (Table 2). The results obtained with SIL and EAG in the standards are lower and with the EAG regarding AST and ALT. (Table 2). The test results are significant preventive and curative (p <0.001) with a protective and a restorative effect. In general the results obtained with the preventive tests are more expressive than curative tests (Table 2). Yields (percentage of protection), are higher than the SIL EAG, and tests with higher protective than curative tests (Graph 1). Test results curative (TC) show that transaminase levels go down, with the

Figure 2: Photograph of rat liver treated with the aqueous extract of Gomphena celosioides, then with CCl4 (lot 6) X40: near the centrolobular veins are normal hepatocytes. Spans hepatocytes (C) and venous sinusoids (D) are clearly visible. On the outskirts there is acidophilia and pyknosis of rare hepatocytes.

Figure 3: Photograph of rat liver treated with CCl4 and silymarin (lot 7) X 40: hepatocellular lesions (acidophilia and pyknosis) are perilobular.

Figure 4: Photograph of rat liver treated with CCl4 and then treated with aqueous extract of Gomphena celosioides (lot 8) X 40: hepatocyte necrosis with a few foci of vacuolar degeneration, the liver remains recognizable.

Figure 5: Photograph of the liver of rats treated with CCl4 and to silymarin (lot 9) X 40: presence of some necrotic cells and vacular...
but there are pockets of vacuolar degeneration and necrosis (Figure 4). As a cure for SIL (Lot 9) the hepatocellular lesions are found where they exist on the periphery of the lobules and are types of vacuolar degeneration (Figure 5). In total the preventive treatment appears to affect EAG more hepatoprotective than curative against CCl4 poisoning.

DISCUSSION

Weight loss of animals observed, is due to the imposed 12-hour fast every day to animals throughout the experiments. This weight loss is compounded by the toxic effects of CCl4. In terms of percentage of relative weights, the values obtained compared to the control groups do not make an assessment in relation to the effects of the tested products. The safety of the plant is justified by the results of the substances assayed after treatment with EAG alone and histological sections. These show in comparison with the standards that the EAG has not led to the phenomena of intoxication in rats as confirmed by the work of Dosumu et al. (2010). Olive oil (HO) used to prepare the solution of intoxication as shown by the results, presented no problem with rats. She may even have a protective effect by causing increased activity of antioxidant enzymes and reduced signs of damage in the liver (Stark and Medar, 2002; Visioli and Galli., 2002; Nakbi et al., 2010).

It is known that CCl4 hepatotoxicity is a dose-dependent. Its toxicity is mainly due to the appearance of free radicals or toxic forms of oxygen that induce lipid peroxidation leading to the destruction of cell membranes (Conso, 2000) The CCl4 hepatotoxicity is also a mandatory action and predictable type indirect (Collat, 1999; Testud, 2005). The increase in serum transaminases and alkaline phosphatase after injection of CCl4, is evidence of significant liver. Liver injury induced by CCl4 (Figure 1), are commonly used as model for drug testing and the extent of liver damage is assessed by the level of cytoplasmic transaminase (ALT and AST) and PAL outstanding (Patrick-Iwuanyanwu et al., 2007; Joshua and Hegde, 2009).

The decrease in liver enzymes by the EAG as shown by the results in Table 2 and Figures 2 and 4, is an indicator of the regeneration process of the repair tissue damage caused by CCl4 liver (Suresh Kumar and Mishra, 2008; Moselhi and Ali, 2009). The results corroborate those of Thabrew et al. (1987), who reported that serum transaminases are restored with the regeneration of hepatocytes and the restructuring of the liver parenchyma. Test results show that preventive and curative treatments to protect the liver EAG and repair damage caused by CCl4. The ability of hepato protective substances to reduce the harm or to preserve the mechanisms of liver function against disturbances of hepato toxin, is an indication of their protective effect (Krishna et al., 2010). Repeated administration of the EAG therefore protects the liver against toxicity caused by CCl4 with efficiency similar to that of SIL. Following lesions induced by CCl4, we are witnessing a substantial increase in values of AST and ALT which is an obvious sign of cell lysis and loss of functional integrity of the membrane of hepatocytes. The decrease in morphological lesions induced by CCl4 is a sign of repair of hepatocytes, increased parenchyma, following treatment with the extract. The decrease in serum AST, ALT and PAL, is a sign of improvement of liver function. If the ALT is the best indicator of poor liver function, total bilirubin (TB) is also a (Gupta et al., 2005). The EAG reduced the rate of BT confirming its protective effects with a yield of 68.25%, and healing with a yield of 44.44%. These returns also confirm its effectiveness in the functioning of liver cells as shown by (Yue et al., 2004, Pal et al., 2006), based on bilirubin, with groups of rats treated with isoniazid. Fleurentin work and Merry, 1990, on the effects of plant extracts hepatoprotective properties, have shown that extracts of Rosmarinus officinalis, and silymarin from Silybum marianum work better in preventative and have no therapeutic effect in acute treatment. This result can be classified in this category Gomphrena celosioides plants and confirm the results obtained with silymarin.

Botha et al. (1986), Vieira et al. (1994), de Moura et al. (2004) revealed the presence of saponins, steroids, amino acids, non-reducing sugars, phenols and flavonoids in Gomphrena celosioides. Flavonoids are known for their hepatoprotective (Seevola et al., 1984; Fintelmann and Wegner, 1999). Antioxidant activities and hepato protective of the EAG, may be due to the presence of flavonoids. Water is a solvent that can extract most of the chemical constituents responsible for various activities under review which justifies the relevance of the traditional use of the plant. Polyphenolic substances soluble in water, with radical-scavenging properties, may also explain the hepato protective properties of the EAG as those of the SIL. Saponins, steroids and triterpenes have liver protective properties (Germano et al., 1999, Germano et al., 2001). It can have a synergistic action between the different chemical constituents soluble in water.

A total of Gomphrena celosioides is a harmless plant in hepato protective effect by the presence of a number of molecules whose mechanisms of action remain to be defined.

REFERENCES


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