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RESEARCH ARTICLE

EVALUATION OF THE HEPATOPROTECTIVE ACTIVITY OF *GOMPHRENA CELOSIOIDES* (AMARANTHACEAE) ON WISTAR RATS INTOXICATED WITH TETRACHLORIDE CARBON

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ABSTRACT

Gomphrena celosioides is a plant used in traditional medicine for treating liver diseases. Tetrachloride Carbon (CCl_4) was used to induce liver toxicity on rats. This hepatotoxicity caused a significant rise in liver enzymes, bilirubin and liver cell damage. The different treatments with aqueous extract of *Gomphrena celosioides* (EAG) at a dose of 500 mg / kg of body weight (BW) and silymarin (SIL) recognized for its hepatotoxic properties at a dose of 300 mg / kg BW decreased levels of these parameters and repaired liver damage. Preventive treatment of animals with EAG and SIL have decreased the rate of serum transaminases, alkaline phosphatase and bilirubin with a yield of 65.06% for EAG and 78.34% for SIL about alanine amino transferase (ALT). Curative treatment of animals with EAG and SIL have a yield of 56.35% to 70.45% against the EAG to SIL about the ALT. Hepatoprotective activity of EAG is more protective than curative and is comparable to SIL's activity. Possible mechanisms for this activity may be due to the action of antioxidants in flavonoids, present in the EAG.

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 the treatment of many diseases (Kubmarawa *et al*., 2007). *Gomphrena celosioides* is an Amaranthaceae. Over of 140 species of the same family exist 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 problems (Onocha *et al*., 2005). In Benin, traditional healers use this plant in the treatment of many diseases including liver diseases, malaria and dysmenorrhea (Adjanohoun *et al*., 1989). Vieira (1994) have demonstrated the analgesic, tonic, carminative and diuretic properties of this plant. Recently, Dosumu *et al.*, (2010) reported its antimicrobial and anti-helminthic properties.

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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 to be undertaken in order 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 plant in comparison with that of silymarin. This study is conducted on Wistar rats whose livers were intoxicated with tetrachloride carbon (CCl_4).

MATERIALS AND METHODS

Materials

63 Wistar rats of both sexes, aged of 8 months with average weight equal to 260 ± 20g, obtained at the International Centre for Research-Development of Animal Husbandry in sub-

humid areas of Bobo-Dioulasso (Burkina-Faso) have been used. These animals were housed in environmental standards conditions, 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 (OCDE, 2008). Plant material consists of freeze-dried stems with leaves of *Gomphrena celosioides* harvested at N'Dali, North East of Benin with 500 kilometers 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 number 6335/HNB AA. Tetrachloride Carbon, provided by UBC.HR. 6172 Leuven Belgium, has been used to induce liver toxicity. The extra virgin olive oil brand Belle France (Francap, BP 30403-75564 Paris Cedex 12) were used for preparation of intoxication. The Legalon ®, Lot B 0902953, manufactured by MADAUS GmbH 51101 Cologne Germany has been 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).

Methods

Experimentations were performed on nine lots of seven rats. Five lots 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 were prepared before each test. The different treatments were done daily and at the same time. The animals were fasted for 12 hours and watered only one hour before handling. They were fed an hour after the manipulations. Twenty-four hours after the last treatment, animals were weighed and anesthetized with ether. Their blood were collected by cardiac puncture into dry tubes and serum. It were used to estimate levels of serum transaminases: aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin (BT) and conjugated bilirubin (CB). Animals were sacrificed and their livers were carefully collected, examined, rinsed with a solution of 10% NaCl, weighed and preserved in 10% formalin for histological studies.

Transaminases dosage (AST, ALT)

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:

AST: L-aspartate + 2-Oxoglutarate → oxaloacetate + L-glutamate oxaloacetate + NADH + H^+ → NAD^+ + L-malate

ALT: alanine + alpha-ceto pyruvic acid glutamate + glutamate → Pyruvic acid + NADH + H^+ → NAD^+ + lactate

Decreasing in absorbance due to conversion of NADH to NAD^+ and proportional to the activity of GOT (or GTP) were measured at 340 nm.

Bilirubin dosage

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

Sulfanilic acid + $NaNO_2$ ⟶ Diazonium

Bilirubin + Diazonium $\xrightarrow{\text{DMSO}}$ compound Diazonium

DMSO dissolved in the aqueous phase unconjugated bilirubin. The determination of direct bilirubin (DB) were done in absence of DMSO.

Direct Bilirubin + diazo ⟶ compound Diazonium

Presence of hydrochloric acid prevented the diazotization of unconjugated bilirubin in the assay without DMSO.

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

Controls

To verify the effects of different substances used for experiments on animals, lots 1, 2, 3, 4, 5, (control groups), were respectively given:

- water (H_2O) per os,
- 0.5 ml Olive oil (HO) intraperitoneally (IP) for 4 days,
- 0.5 ml of Tetrachloride carbon (CCl_4) per kg by IP for 4 days (Kamssouloum, 1984);
- 500mg/kg of the aqueous extract of *Gomphrena celosioides* (EAG) orally given for 5 days;
- 300mg/kg of silymarin (SIL) orally given for 5 days.

Preventive treatment

Preventive therapy (PT) highlighted the preventive properties of EAG in comparison with that of SIL on lots 6 and 7. The rats of lot 6 each received orally 500 mg / kg of EAG for 5 days followed by 0.5 ml / kg of CCl_4 in IP for 4 days. The rats of lot 7 received orally 300 mg / kg of SIL for 5 days, then 0.5 ml / kg of CCl_4 in IP for 4 days.

Cure

The cure (TC) highlighted the healing properties of EAG in comparison with that of SIL on lots 8 and 9. The rats of lot 8 received by IP, 0.5 ml / kg of CCl_4 for 4 days. Then they were orally given 500 mg / kg of EAG for 5 days. The rats of lot 9 received orally 0.5 ml / kg of CCl_4 for 4 days and 300 mg / kg of SIL for 5 days.

Processing and data analyses

Data entry were performed using Excel 2007.

- Calculation of relative weights (RW)

RW= Liver Weight/ Body weight x 100

- Calculation of the percentage of protection (Performance)

% protection = 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

Morphometric parameters

Table 1 shows the effects of various treatments on body weight and liver weight on Wistar rats and the comparison of means (by level of treatment.

pronounced in animals which received CCl_4 treatment. The largest decreases were observed in animals that received CCl_4 only. The results of animals given preventive treatment and curative treatment are significant ($p < 0.001$). The percentages of relative liver weight ranged from 4.24 to 6.11 ± 0.66% ± 0.44% (Table 1). These values are significant ($p < 0.001$). They are very different out from each other for preventive and curative treatments.

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)

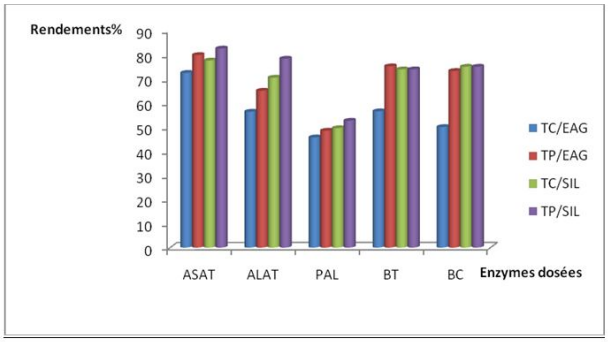
Treatments	d_weight	P_liver	P_rel
	***	***	***
H_2O	4.29^{d}±1.80	13.71^{a}±1.80	5.63^{ab}±0.71
HO	4.86^{d}±0.38	14.86^{a}±1.07	6.11^{a}±0.44
CCL_4	12.00^{a}±0.58	10.00^{b}±1.63	4.24^{c}±0.66
SIL	8.00^{c}±1.15	13.71^{a}±1.80	5.70^{ab}±0.74
EAG	8.14^{c}±0.70	13.71^{a}±1.80	5.70^{ab}±0.73
SIL_CCL_4	8.29^{bc}±0.76	14.29^{a}±1.80	5.99^{a}±0.75
EAG_CCL4	8.57^{bc}±0.98	13.42^{a}±1.90	5.68^{ab}±0.80
CCL_4_SIL	9.71^{b}±0.76	12.57^{a}±1.51	5.34^{abc}±0.64
CCL_4_EAG	9.86^{b}±0.38	12.00^{ab}±1.63	5.06^{b}±0.69

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

Traitement	ASAT	ALAT	PAL	BT	BC
	***	***	***	***	***
H_2O	38.43^{cde}±1.51	41.14^{ef}±2.19	80.00^{b}±27.54	6.00^{c}±1.73	2.00^{bc}±0.82
HO	37.86^{de}±4.10	41.57^{e}±3.26	78.43^{b}±27.45	5.00^{c}±1.41	1.00^{c}±0.82
CCl_4	200.71^{a}±13.66	172.14^{a}±13.83	148.57^{a}±22.55	18.00^{a}±5.07	6.00^{a}±1.73
SIL	45.00^{c}±1.73	50.86^{d}±1.57	75.00^{b}±1.63	6.00^{c}±1.29	2.00^{bc}±1.29
EAG	38.42^{cde}±2.37	39.86^{ef}±2.60	75.00^{b}±19.10	6.00^{c}±1.15	2.00^{bc}±0.82
SIL_CCl_4	35.00^{de}±1.15	37.29^{ef}±1.80	70.43^{b}±15.54	6.00^{c}±0.82	2.00^{bc}±1.00
EAG_CCl_4	40.43^{cd}±2.37	60.14^{c}±1.77	76.57^{b}±18.61	5.71^{c}±1.38	2.14^{bc}±0.90
CCl_4_SIL	45.00^{c}±1.73	50.86^{d}±1.57	75.00^{b}±1.63	6.00^{c}±1.29	2.00^{bc}±1.29
CCl_4_EAG	55.29^{b}±1.11	75.14^{b}±2.60	80.71^{b}±1.80	10.00^{b}±2.16	4.00^{b}±216

***= 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

TC / EAG = curative treatment with the aqueous extract of Gomphrena TP / EAG = Preventive treatment with the aqueous extract of Gomphrena
TC / SIL = curative treatment with silymarin TP / SIL = Preventive treatment with silymarin Rendements = Yields

Monitoring of body mass of animals during the different treatments shows a significant weight loss. The smallest decline of average weight is 4.29 ± 1.80 g and the largest decrease was 12.00 ± 0.50 g. This weight loss is more

Biochemical parameters

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 H_2O and those who received only HO are in compliance with standards which are: 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. Results were very high with animals which received CCl_4 only. (Table 2). The results obtained with SIL and EAG in the standards are lower. (Table 2). The test results are significantly 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) of SIL are higher than EAG, and tests were higher protective than curative tests (Graph 1). Curative Test results (TC) showed that transaminases levels decreased with the administration of EAG at 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 protectors test results (TP) pointed out that with the administration of the EAG there is a hepatoprotective activity significantly comparable to that of SIL with a protection level from 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 CCl_4. 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 lot 1 rats which is normal control, shows a normal lobular architecture, marked by the presence of hepatocellular spans arranged around a central vein (CV).These bays are separated by sinusoids. In animals treated with the HO (lot 2), with EAG (lot 4) and treated with SIL (lot 5), the hepatic architecture is generally preserved. In animals of lot 3, poisoned with CCl_4, the trabecular organization of the liver is unrecognizable (Figure 1). There is a massive hepatocyte necrosis with centrilobular vacuolar degeneration, a caryopycnose, a karyolysis and cytoplasmic acidophilia predominantly perilobular. That hepatocyte necrosis is accompanied by congestion of sinusoids and dilated centrilobular veins. After administering a preventive treatment to the EAG for 5 days, followed by CCl_4 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 centrolobular veins are nearly to normal hepatocytes (Figure 2). Rats of lot 7 have received preventive treatment with SIL for 5 days followed by CCl_4 intoxication (Figure 3). Liver lesions observed are faint and just a few hepatocytes acidophilia are on perilobular region. After the cure for the EAG animals poisoned by CCl_4 (lot 8), hepatocyte necrosis observed is less important than in animals intoxicated and untreated. The liver is generally recognizable 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 CCl_4 poisoning.

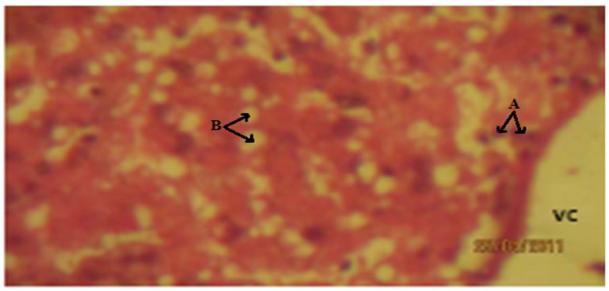


Figure 1: Photography of the liver of rats treated with CCl_4 (Lot 3) X 40: massive hepatocyte necrosis (pyknotic nucleus (A)) with predominantly centrilobular vacuolar degeneration (B).

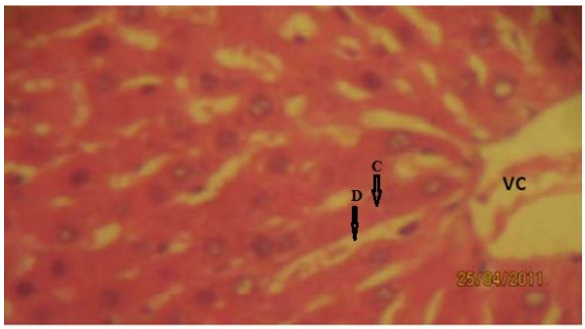


Figure 2: Photography of rat liver treated with the aqueous extract of *Gomphena celosioides*, then with CCl_4 (lot 6) X40: near the centrolobular veins are normal hepatocytes. Spans hepatocytes (C) and venous sinusoids (D) are clearly visible. On the outskirts there is acidophilia and pyknosis of rare hepatocytes.

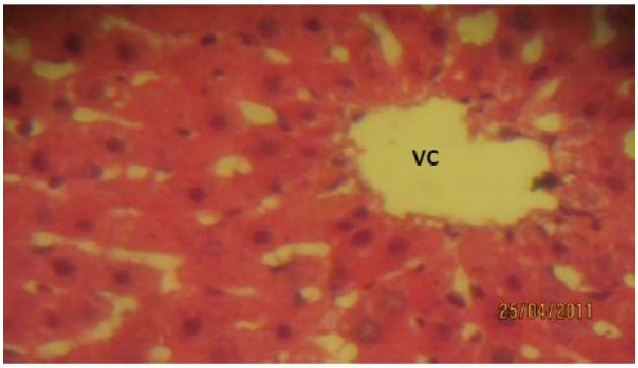


Figure 3: Photography of rat liver treated with CCl_4 and silymarin (lot 7) X 40:hepatocellular lesions (acidophilia and pyknosis) are perilobular

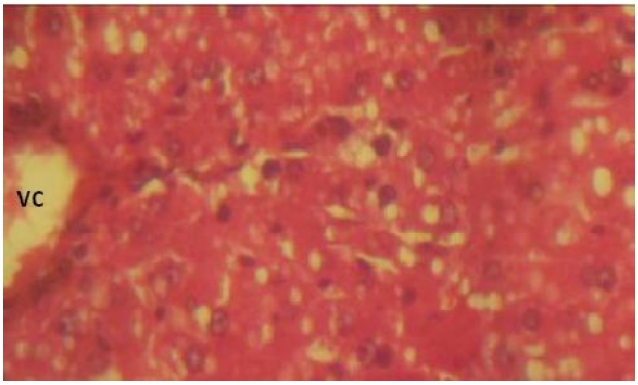


Figure 4: Photography of rat liver treated with CCl_4 and then treated with aqueous extract of *Gomphena celosioides* (lot 8) X 40: hepatocyte necrosis with a few foci of vacuolar degeneration, the liver remains recognizable

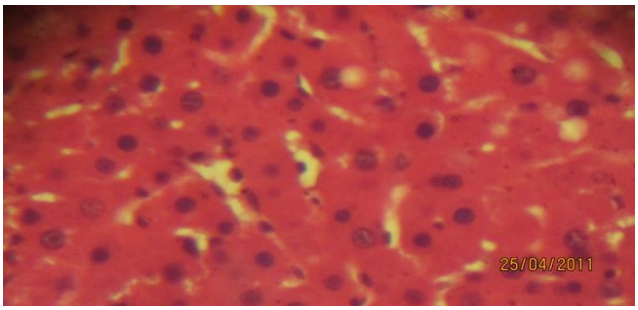


Figure 5: Photography of the liver of rats treated with CCl_4 and to silymarin (lot 9) X 40: presence of some necrotic cells and vacuolar

DISCUSSION

Loss weighting 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 CCl_4. 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 (Nakbi *et al*., 2010; Stark and Medar, 2002; Visioli and Galli., 2002). It is known that CCl_4 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 CCl_4 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 CCl_4, is evidence of significant liver. Liver injury induced by CCl_4 (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; Joshia 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 CCl_4 liver (Suresh 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 CCl_4. The ability of hepatoprotective substances to reduce the harm or to preserve the mechanisms of liver function against disturbances of hepatic toxin, is an indication of their protective effect (Krishna *et al.,* 2010). Repeated administration of the EAG therefore protect the liver against toxicity caused by CCl_4 with an efficiency similar to that of SIL. Following lesions induced by CCl_4, 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 CCl_4 is a sign of hepatocytes repairing, 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 results 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 by isoniazid . Fleurentin and Merry's (1990) who worked on the effects of extracts plant with 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; Wegner and Fintelmamann, 1999). Antioxidant and hepatoprotective activities 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. The presence of 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, sterols and triterpenes have liver protective properties (Germanò *et al*., 1999, Germanò *et al.,* 2001). It can have a synergistic action between the different chemical constituents soluble in water. *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.

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