Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 4, Issue, 05, pp.067-072, May, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

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

Maxime Machioud SANGARE[1]; Jean Robert KLOTOE[2]; Victorien DOUGNON*[2,3]; Jean-Marc ATEGBO[1]; Anatole LALEYE[4]; Patrick EDORH[3,5]; Lauris FAH[2]; Maximin SENOU[2]; Frédéric LOKO[2]; Karim Laye DRAMANE[1]

[1]Faculty of Science and Technology, Laboratory of Physiology-Pharmacology, University of Abomey-Calavi, 01 BP 526 Cotonou, Benin.
[2]Polytechnic School of Abomey-Calavi, Research Laboratory in Applied Biology, University of Abomey-Calavi, 01 BP 2009 Cotonou, Benin.
[3]Interfaculty Center of Formation and Research in Environment for the Sustainable Development, Laboratory of Toxicology and Environmental Health, University of Abomey-Calavi (UAC), 01 BP 1463 Cotonou, Benin.
[4]Faculty of Health Sciences, Human Biology Unit, Laboratory of Histology, University of Abomey-Calavi, 01 BP 188 Cotonou, Benin.
[5]Faculty of Science and Technology, Department of Biochemistry and Cellular Biology, University of Abomey-Calavi (UAC), 01 BP 526 Cotonou, Benin.

ARTICLE INFO

Article History:
Received 19th February, 2012
Received in revised form
26th March, 2012
Accepted 25th April, 2012
Published online 30th May, 2012

Key words:
Liver disorders, silymarin, traditional medicine, Hepatotoxicity, flavonoids.

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 treatment of many diseases (Kubmarawa *et al*., 2007). *Gomphrena celosioides* is an Amaranthaceae. Over of 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 helminthic 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).

***Corresponding author:** victorien88@hotmail.com

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 N'Dali, 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 anesthesia with ether. Their blood were 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 (BT) and conjugated bilirubin (BC). The animals were sacrificed and their liver is carefully collected, examined, rinsed with a solution of 10% NaCl weighed and preserved in 10% formalin for histological studies.

Transaminase dosage (GOT, GPT)

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: 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

The decrease in absorbance due to conversion of NADH to NAD^+ and proportional to the activity of GOT (or GTP) 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.

Sulfanilic acid + NaNO2 ⟶ Diazonium

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

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

Direct Bilirubin + diazo ⟶ compound Diazonium

Presence of hydrochloric acid prevents the diazotization of 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 (H_2O) 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)
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

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

1.80 g and the largest decrease was 12.00 ± 0.50 g. This weight loss is more pronounced in animals which contains CCl4 treatment. The largest decreases were observed in animals that received only CCl4. 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 out different 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)

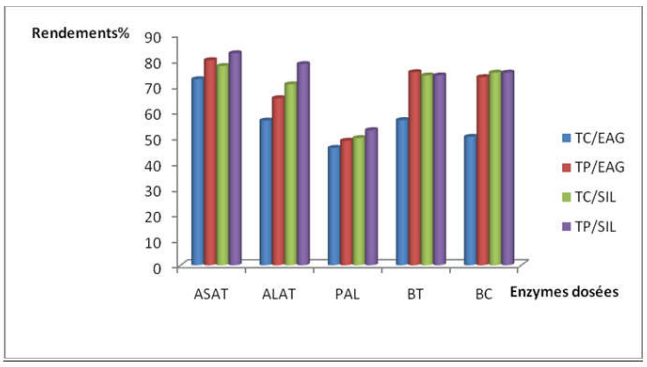
Treatments	d_weight	P_liver	P_rel
	***	***	***
H_2O	4.29^{d}±1.80	13.71^{a}±1.80	5.63^{ab}±0.71
HO	4.86^{d}±0.38	14.86^{a}±1.07	6.11^{a}±0.44
CCL_4	12.00^{a}±0.58	10.00^{b}±1.63	4.24^{c}±0.66
SIL	8.00^{c}±1.15	13.71^{a}±1.80	5.70^{ab}±0.74
EAG	8.14^{c}±0.70	13.71^{a}±1.80	5.70^{ab}±0.73
SIL_CCL_4	8.29^{bc}±0.76	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 ratsand 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^{c}±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
CCl4_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

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. Monitoring of body mass of animals during the different treatments shows a significant weight loss. The smallest decline of average weight is 4.29 ±

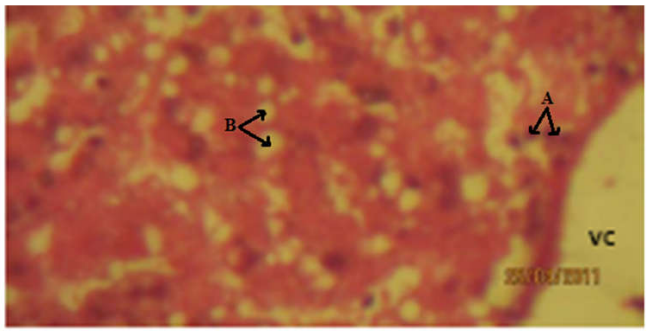


Figure 1: Photograph of the liver of rats treated with CCl4 (Lot 3) X 40: massive hepatocyte necrosis (pyknotic nucleus (A)) with predominantly centrilobular vacuolar degeneration (B).

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 H2O 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. Against

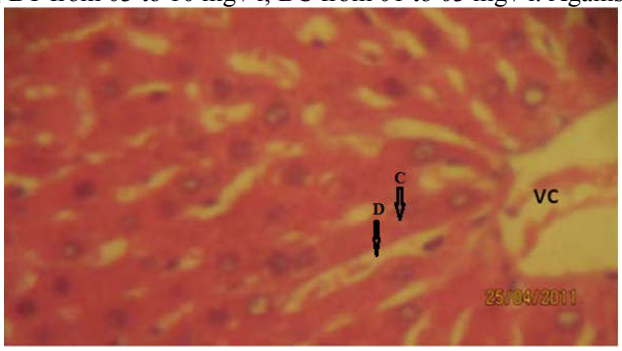


Figure 2: Photograph of rat liver treated with the aqueous extract of Gomphenacelosioides, then with CCl4 (lot 6) X40: near the centrolobular veins are normal hepatocytes. Spans hepatocytes (C) and venous sinusoids (D) are clearly visible. On the outskirts there is acidophilia and pyknosis of rare hepatocytes.

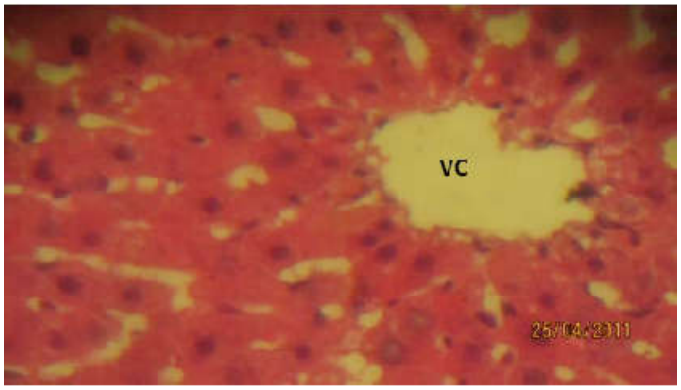


Figure 3: Photograph of rat liver treated with CCl 4 and silymarin (lot 7) X 40:hepatocellular lesions (acidophilia and pyknosis) are perilobular

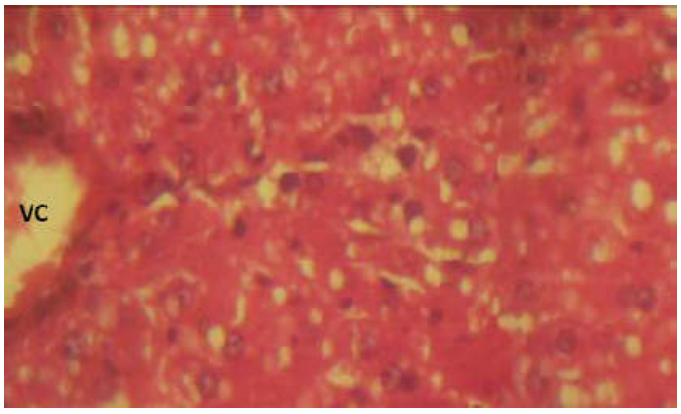


Figure 4: Photograph of rat liver treated with CCl4 and then treated with aqueous extract of *Gomphena celosioides* (lot 8) X 40: hepatocyte necrosis with a few foci of vacuolar degeneration, the liver remains recognizable

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 the EAG, and tests with higher protective than curative tests (Graph 1). Test results curative (TC) show that transaminase levels go down, with the 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

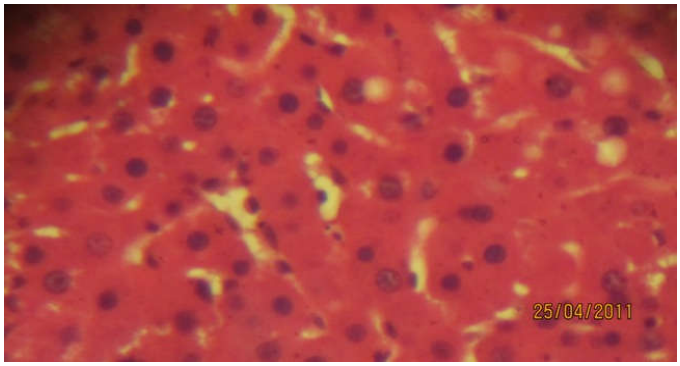


Figure 5: Photograph of the liver of rats treated with CCl4 and to silymarin (lot 9) X 40: presence of some necrotic cells and vacuolar

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 centrilobular vacuolar degeneration, a caryopycnose, a karyolysis and cytoplasmic acidophilia predominantly périlobulaire. That hepatocyte necrosis is accompanied by congestion of sinusoids and centrilobular 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 centrolobular 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

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; 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 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; Fintelmamann 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, sterols 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

Adjanohoun EJ, Adjakidjè V, Ahyi MRA, Ake Assi L, Akoegninou A, d'Almeida J, Apovo F, Boukef K, Chadare M, Dramane K, Eyme J, Gassita JN, Gbaguidi N, Goudoté E, Guinko S, Houngnon P, Issa L, Keita A, Kiniffo H, Koné-Bamba D, Musampa Nseyya A, Saadou M, Sodogandji T, de Souza S, Tchabi A, Zinsou Dossa C, Zohoun T. 1989. Médecine traditionnelle et pharmacopée. Contribution aux études ethnobotaniques et floristiques en République Populaire du Bénin. Agence de Coopération Culturelle et Technique, p 895 : 713-724.

Botha S, Gerritsma-Van der Vijer LM. 1986. Pharmcochmical study of *Gomphrena celosioides* (Amaranthaceae). *Suid-Afrikaanse Tydskrif vir Natuurw-etenskap en Tegnologie*, 5(1): 40-45.

Burkill HM 1984. The useful plants of West Tropical Africa vol 1. Families A-D. Royal Botanical Garden kew. pp. 441-415.

Calzada F, Yepez-Mulia L, Tapia-Contreras A. 2007. Effect of Mexican medecinal plant used to treat tricomoniasis on *Trichomonas vaginalis* trophozoites. *J. Ethnopharmacol*., 113: 248-251.

Collat C. 1999. Hépatite et travail: foie et toxiques d'origine professionnelle. http://www.hepatitis.org/hepaetravail_ fr.htm (Visité le 1er octobre 2011).

Conso F. 2000. Dérivés halogénés des hydrocarbures. *In Bismut C.* Ed. Toxicologie clinique. 5e éd. Paris: Médecine-Sciences Flamarion. P 802.

de Moura RM, Pereira PS, Januário AH, França Sde C, Dias DA. 2004. Antimicrobial screening and quantitative determination of benzoic acid derivative of *Gomprena celosioides* by TLC-densitometry. *Chem. Pharm. Bull.,* 52(11): 1342-1344.

Dosumu OO, Idowu PA, Onocha PA, Ekundayo O. 2010. Isolation of 3-(4-Hydroxyphenyl) Methylpropenoate and bioactivity evaluation of Gomphrena celosioides. *EXCLI J*., 9: 173-180.

Fleurentin J, Joyeux M.1990. Les tests *in vivo* et *in vitro* dans l'évaluation des propriétés anti-hépatotoxiques de substances d'origine naturelle. Actes du 1er Colloque Européen d'Ethnopharmacologie. Ed. ORSTOM., 248-269.

Germanò MP, D'Angelo V, Sanogo R, Morabito A, Pergolizzi S, De Pasquale R. 2001. Hepatoprotective activity of *Trichilia roka* on carbon tetrachloride-induced liver damage in rats, *J. Pharm. Pharmacol.,* 53(11): 1569-1574.

Germanò MP, Sanogo R, Costa C, Fulco R, D'Angelo V, Torre EA, Viscomi MG, De Pasquale R. 1999. Hepatoprotective properties in the rat of *Mitracarpus scaber* (Rubiaceae). *J Pharm. Pharmacol.,* 51(6): 729 – 734.

Gessler MC, Nkunya MH, Mwasumbi LB, Heinrich M, Tanner M. 1994. Screening Tanzanian medicinal plants for antimalarial activity. *Acta Trop.,* 56(1): 65-77.

Gupta RK, Kesari AN, Watal G, Murthy PS, Chandra R, Tandon V. 2005. Nutritional and hypoglycemic Effect of Fruit Pulp of *Annona squamosa* in normal healthy and alloxan-Induced diabetic Rabbits. *Ann. Nutr. Metab.,* 49(6): 407–413.

Hegde K, Joshia AB. 2009. Hepatoprotective effect of *Carissa carandas* root extract against CCl4 and paracetamol induced hepatic oxidative stress. *India J. Exp. Biol*., 47(8): 660-667.

Islam R, Alam AH, Rahman BM, Salam KA, Hossain A, Baki A, Sadik G. 2007. Toxicological studies of two compounds isolated from *Loranthus globosus* Roxb. *Pak. J. Biol. Sci.,* 10(12): 2073-2077.

Krishna KL, Mruthunjaya K, Patel JA. 2010. Antioxidant and hepatoprotective potential of stem methanolic extract of *Justicia gendarussa* Burm. *Int. J. Pharmacol*., 6: 72-80.

Kamssouloum A. 1984, Contribution à l'étude de l'action hépétoprotectrice de Tinospora bakis Menispermaceae (arguments biochimique, histologique, pharmacologiques) Thèse de pharmacie, UCAD,Dakar : p 126.

Kubmarawa D, Ajoku GA, Enwerem NM, Okorie DA, 2007. Preliminary phytochimical and antimicrobial screening of 50 medecinal plants from Nigeria, *Afr. J. Biotechnol*., 6(14): 1690-1696.

Moselhy SS, Ali HK. 2009. Hepatoprotective effect of cinnamon extracts against carbon tetrachloride induced oxidative stress and liver injury in rats. *Biol. Res.,* 42(1):93-98.

Nakbi A, Tayeb W, Grissa A, Issaoui M, Dabbou S, Chargui I, Ellouz M, Miled A, Hammami M. 2010. Effects of olive oil and its fractions on oxidative stress and the liver's fatty acid composition in 2, Dichlorophenoxyacetic acid-treated rats. *Nutr. Metab.,* 7:80

OCDE. (Organisation de Développement et de Coopération Economique). 2008. Lignes directrices de l'OCDE pour les essaies de produits chimiques. Toxicité orale aiguë-Méthode de l'ajustement des doses. Essai n° 425 DOI: 10.1787/9789264071056-fr

Onocha PA, Ajaiyeoban EO, Dosumu OO, Ekundayo O. 2005. Phytochemical Screening and Biological Activities of *Gomphrena celosioides* (C.Mart) extracts. *Nigerian Soc. Exp. Biol. J.,* 5(2): 59-65.

Pal R, Vaiphei K, Sikander A, Singh K , Rana SV. 2006. Effect of garlic on isoniazid and rifampicin-induced hepatic injury in rats. *World J. Gastroenterol*., 12(4): 636-639.

Patrick-Iwuanyawu KC, Wegwu MO, Ayalogu EO. 2007. Prevention of CCl4- induced liver damage by ginger, garlic and vitmin E. *Pak J. Biol. Sci*., 10(4): 617-621.

Rauen HM, Schriewer H. 1971. The antihepatotoxic effect of silymarin on liver damage in rats induced by carbon tetrachloride, d-galactosamine and allyl alcohol. Arzneimittelforschung 21(8) : 1194-1201.

Seevola D, Barbarini G, Grosso A, Bona S, Perissoud D. 1984. Flavonoids and hepatic cyclic monophosphates in liver injury. *Boll. Ist. Sieroter. Milan*., 63(1): 77-82.

Stark AH, Madar Z. 2002. Olive oil as a functional food: epidemiology and nutritional approaches. *Nutr. Rev*., 60(6):170-176.

Suresh Kumar SV, Mishra SH. 2008. Hepatoprotective effect of *Pergularia daemia* (Forsk.) ethanol extract and it fraction. *Indian J. Exp. Biol.,* 46(6): 447-452.

Testud F. 2005. Pathologie toxique professionnelle et environnementale. Editions Aska : Paris, p.672.

Thabrew MI, Joice PD, Rajatissa W. 1 987. A comparative study of efficacy of *Pavetta indica* and *Osbeckia octandra* in the treat ment of liver dysfunction. *Planta Med.,* 53 (3): 239-241.

Vieira CCJ, Mercier H, Chu EP, Figueiredo-Ribeiro RCL. 1994. *Gomphrena* species (globe amaranth) *in vitro* culture and production of secondary metabolites. In: Bajaj, Y.P.S., ed. *Biotechnol. Agr. Forest.*: Medicinal and Aromatic Plants VII. 28: 257-270.

Visioli F, Galli C. 2002. Biological properties of olive oil phytochimicals. *Crit. Rev. Food Sci. Nutr.,* 42(3):209-221.

Wagner WL, Herbst, DR, Sohmer SH. 1999. Colocasia. In: Manual of the Flowering Plants of Hawaii. University of Hawaii *Press*, Honolulu, Hawaii, 1356-1357.

Wegner T, Fintelmamann V. 1999. Pharmacological properties and therapeutic profile of artichoke (Cynara scolymus L.). *Wien Med Wochenschr.*149(8-10): 241-247.

Yue J, Peng RX, Yang J, Kong R, Liu J. 2004. CYP2E1 mediated isoniazid-induced hepatotoxicity in rats. *Acta Pharmacol Sin.,* 25(5):699-704.

****