EVALUATION OF THE HEPATOPROTective ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS FROM GOMPHRENA CELESIOIDES, COLA NITIDA AND ENTENDROPHRAGMA ANGOlENSE AGAINST CCL4 INDUCED HEPATOTOXICITY IN RATS.

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ABSTRACT

Entandrophragma angolense, Cola nitida and Gomphrena celosioides are plants used by traditional Ivorian medicine for the treatment of several diseases including liver disease. The aqueous and ethanol extracts of these plants were the subject of several studies around the world, however, there is still much to do for the elucidation of their hepatoprotective properties. The point of this study was to evaluate the hepatoprotective properties of extracts of these three plants. The extractions were carried out by maceration. Hundred (100) g dry powder of plant in 2 liters of distilled water (aqueous extraction) or in 2 liters of water/ethanol mixture (30/70 V/V) (ethanol extraction). As regards the hepatoprotective properties, Rats were pretreated with the extract (200 and 500 mg/kg) by silymarin (100mg / kg) for a week. Then, liver toxicity was induced by carbon tetrachloride 20% (5ml/kg) on the seventh day. The results showed that the extracts studied, lower the effect of CCl4 on biochemical parameters, reducing (P<0.05) serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), the alkaline phosphatase (ALP), gamma-glutamyl transferase (γ-GT) and total bilirubin. This study reveals that the studied extracts possess more interesting...
hepatoprotective properties for ethanol extracts at 500mg / kg. The ethanolic extracts of C.nitida, and E.angolense at 500 mg /kg give results comparable to that of silymarin.

KEYWORDS: Gomphrena, Entandrophragma, Cola, hepatotoxicity, silymarin, CCl4.

INTRODUCTION

In Africa, liver diseases are common and are a real human health issue. Alcoholism, bacterial or viral hepatitis, aflatoxin and self-medication are often contributing factors.[1] These liver diseases require lengthy and expensive hospital stays. Which makes about 80% of the rural population rely on traditional medicine for their primary healthcare needs.[2] Medicinal plants are widely used for the prevention and treatment of various diseases in Africa and in developing countries.[3] It is therefore appropriate to carry out pharmacological research to study the effectiveness of the plants used in traditional medicine and monitor their safety, to enable more efficient use.

Cola nitida, Gomphrena celosioides and Entandrophragma angolense are plants traditionally used by people to treat several diseases, among others hepatitis, malaria, gastric ulcer, inflammation and anemia. Scientifically, more study was done on the biological properties of various extracts of these plants. We note that Gomphrena celosioides showed anti-bacterial, anti-viral[4] and antioxidant.[5] Properties Cola nitida proved to be an antimicrobial[6] and showed antioxidant properties.[7] Entandrophragma angolense would have antiplasmodial[8], and antianemic[9] properties. We now understand that multiple studies, certainly dispersed, were made on these medicinal plants. What denote all the emphasis on the use of traditional medicine plants in world health policy. However, available information on the hepatoprotective properties of these plants are limited.

This study aims to determine the hepatoprotective properties of aqueous and ethanolic extracts from Cola nitida, Gomphrena celosioides, and Entandrophragma angolense with the Wistar rat; in order to justify the traditional use of this plant in the treatment of liver diseases.

MATERIAL AND METHODS

Plant material

The plant material consists of Gomphrena celosioides (stems, leaves, flowers), Cola nitida (fruits) and Entandrophragma angolense (bark). A sample of each plant was authenticated by
the Laboratory of Botany and Plant Biology of the Biosciences Training and Research Unit (Félix Houphouet Boigny University).

**Animal Material**

Four ninety albino Wistar strain of rats, average weight 142.618 g were used for experiments. They were fed with standard rodent diet and treated according to the guidelines of the for Economic Cooperation and Development Organisation.\[^{10}\]

**Preparation of aqueous and ethanol extracts**

Plants were air dried for two weeks and then ground. Aqueous extraction was made according to the method described by.\[^{11}\] Distilled water was used for aqueous extraction and water / ethanol mixture (30/70 v/v) for ethanol extraction

**Treatment of animals.**

Determination of hepatoprotective properties of the extracts was performed according to the method described by\[^{12}\] with some modifications. The carbon tetrachloride is diluted to 20% with paraffin oil. Silymarin, extracts and NaCl are administered by gavage for seven days; CCL4 and paraffin intraperitoneally for seven days. The animals were weighed daily and sacrificed on the eighth day. The experiments were performed on 15 batches of 6 rats and each batch received the following treatments.

T: NaCl 9 % at 5 ml / kg and paraffin 5ml / kg.
TN: NaCl 9 % at 5 ml / kg and CCl4 at 5ml / kg.
TP: SIL at 100 mg / kg in 5 ml / kg and CCl4 at 5ml / kg.
HEG1: 200 mg/kg of ethanolic extract of *Gomphrena celosioides* at 5 ml/kg, and CCl4 at 5 ml/ kg.
HEG2: 500 mg / kg of ethanolic extract of *Gomphrena celosioides* at 5 ml/kg, and CCl4 at 5 ml/kg.
HAG1: 200 mg / kg of aqueous extract of *Gomphrena celosioides* at 5 ml/kg, and CCl4 at 5 ml/kg.
HAG2: 500 mg / kg of aqueous extract of *Gomphrena celosioides* at 5 ml/kg, and CCl4 at 5 ml/kg.
HEE1: 200 mg/kg of ethanolic extract of *Cola nitida* at 5 ml/kg, and CCl4 at 5ml/kg.
HEE2: 500 mg/kg of ethanolic extract of *Cola nitida* with 5 ml/kg, and CCl4 at 5ml/kg.
HAE1: 200 mg/kg of aqueous extract of *Cola nitida* 5 ml/kg, and CCl4 at 5ml/kg.
HAE2: 500 mg/kg of aqueous extract of *Cola nitida* 5 ml/kg and CCl4 at 5ml/kg.
HEY1: 200 mg/kg of ethanolic extract of *Entandrophragma angolense* at 5ml/kg, and CCl4 at 5ml/kg.
HEY2: 500 mg/kg ethanolic extract of *Entandrophragma angolense* at 5ml/kg, and CCl4 at 5 ml/kg.
HAY1: 200 mg/kg of aqueous extract of *Entandrophragma angolense* at 5ml/kg, and CCl4 at 5 ml/kg.
HAY2: 500 mg/kg of aqueous extract of *Entandrophragma angolense* at 5ml/kg, and CCl4 at 5ml/kg.
Sample
The animals were euthanized 24 hours after the last treatment. The blood of each animal was
taken (puncture of the orbital sine) in a tube without anticoagulant, in order to proportion the
biochemical parameters.

Biochemical study
The serums obtained after centrifugation, were used to proportion the biochemical parameters
that is: alanine aminotransferase (ALAT), the aspartate aminotransferase (ASAT), Gamma-
Glutamyl Transferase (γ-GT), Alkalin Phosphatases (PAL) and total bilirubin, with an
automate of Cobas type C311 HITACHI of ROCHE (France).

Data processing and analyses
The statistical analysis of the results was carried out by using the analysis of variances
(ANOVA ONE WAY) of the software Graph Pad PRISM 5.0 (Microsoft USA). The
differences between the averages were given according to the Tukey multiple comparison
Tests. These differences are considered significant when p is lower than 0.05 (p < 0.05).

RESULTS

Figure 1: Effect of extracts of silymarin on the enzyme activity of ASAT.
Figure 2: Effect of extracts of silymarin on the enzyme activity of ALAT.

Figure 3: Effect of the extracts and silymarin on the enzymatic activity of γ-GT

Figure 4: Effect of the extracts and silymarin on the enzymatic activity of PAL
Figure 5: Effect of the extracts and of silymarin on the concentration of total bilirubin.

Values are average ± E.S.M (Standard error of the average) with n = 6. * P <0.05; ** P <0.01; *** P <0.001: significantly different from the batch TN. # P <0.05; ## P <0.01; ### P <0.001: significantly different from the batch TP.

**DISCUSSION**

Carbon tetrachloride is an experimental hepatotoxic widely used. Its toxicity is mainly due to the appearance of free radicals or toxic oxygen species that induce lipid peroxidation leading to the destruction of cell membranes.\[13\] This is why intraperitoneal injection of CCl4 to animals, causes an intoxication of the liver. This liver intoxication has resulted in a significant increase in the serum rate of transaminases (fig 1-2) of the gamma-glutamyl transferase (fig 3), alkaline phosphatase (Fig 4) and total bilirubin (fig 5); this of course in TN batch compared to T and TP Batch. The Increase in levels of these biochemical parameters in serum is evidence that the CCl4 caused hepatocellular lesions, one of the consequences of which is the release of intracellular enzymes following the destruction of liver cells. Indeed, when the membrane of liver cells is damaged, a variety of enzymes, usually localized in the cytosol are released in the bloodstream. Their estimation in serum is a useful measure of the quantitative marker and type of liver cell damage.\[14\] The liver injuries induced by CCl4 are commonly used as a model for screening for hepatic drugs and the extent of damage is evaluated by the level of cytoplasmic transaminases (ALT and AST) and PAL prevailing.\[15\] Note that the CCl4 like any other hepatotoxic substances, decreases the ability of the liver to synthesize albumin.\[16\]
Treatment of animals by aqueous and ethanolic extracts of *G.celosioides, C.nitida,* and *E.angolense* has enabled a significantly lower levels of markers mentioned above. Changes in serum concentrations of each of these parameters are related to the functioning of liver cells.[17] The properties of our extracts proved more important at a dose of 500 mg / kg of Pc, more interesting for ethanol extracts and greater efficiency for the ethanol extract of *Cola nitida*. These new trends in liver enzymes rate caused by aqueous and ethanol extracts of *G.celosioides, C.nitida,* and *E.angolense* are indicators of the regeneration of the repair process of liver tissue damage due to CCl4.[18] These results are consistent with the work that has reported that serum of transaminases are restored with the regeneration of hepatocytes and restructuring of the liver parenchyma.[19] Results comparable to those obtained with our extracts have also been reported by.[20] This showed the antioxidant and hepatoprotective potential of total extracts rich in phenolic compounds.

The treating of rats with extracts after CCl4 intoxication explicitly shows that the aqueous and ethanolic extracts of *G.celosioides, C.nitida,* and *E.angolense* protect the liver and repair damage caused by carbon tetrachloride. And also that our extracts have not been able to fully repair the hepatocyte injuries induced by CCl4 but could limit them.[21] For our extracts led to enzyme concentrations still statistically different from the lot of intoxicated controls and treated by silymarin. The ability of hepatoprotective substances to reduce the damages or preserve functioning mechanisms of liver against disruption of hepatotoxin, is a mark of its protective effect.[22]

The toxicity of CCl4 being essentially due to the appearance of free radicals or toxic oxygen species.[13] So we imagine that a substance could have hepatoprotective properties if it has antioxidant properties. Several authors have shown that the different types of chemical compounds identified in the plant extracts have therapeutic effects. These are sterols and triterpenes used for their antipyretic and analgesic properties.[23] There are also glycosides and terpene sterol for their anti-inflammatory activity, coumarins which are anticoagulants; they are also sedative, hypnotic, anticonvulsant, antispasmodic and hypotensive.[24] There are also flavonoids known for their hepatoprotective activities[25], their ability to reduce blood pressure and protect the liver.[26,27] has revealed some spermicide, analgesic, and immunomodulating, saponosides cytoprotective and anti-bacterial and laxative properties of reducing compounds.[28] showed that the extracts rich in phenols and flavonoids present great antioxidant potential in vivo and hepatoprotective activity against the damages induced by
CCl4. We deduce then that the extracts studied certainly owe their hepatoprotective properties to the phytochemicals compounds they possess particularly phenols and flavonoids.

4. CONCLUSION
Our study was to provide scientific support to the traditional use of the ethanol extract of Gomphrenaceelosioides as treatment for liver infections by our population. This work showed that the studied extracts reduce the toxic effects of CCl4 on the liver of rats. Indeed, treatment with these extracts significantly decreased serum concentrations of transaminase (ALAT, ASAT), gamma-glutamyl transferase, alkaline phosphatase and total bilirubin. These hepatoprotective properties have proved to be more interesting to the dose of 500 mg / kg of PC, compared to a dose of 200 mg / kg bw; with better activity for the ethanol extract of Cola nitida and Entandrophragma angolense.

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REFERENCES


