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Antihepatotoxic Activity of *Xylopia phloiodora* Extracts on Some Experimental Models of Liver Injury in Rats

[1]Paul F. Moundipa, [2]Silvère Ngouéla, [3]Georges Alain Tchamba, [1]Nico F. Njayou,
[1]Pascal D.D. Chuisseu, [2]Fabien Zéléfack and [2]Etienne Tsamo
[1]Department of Biochemical Pharmacology and Toxicology,
[2]Laboratory of Organic Chemistry, University of Yaounde I, P.O. Box 812 Yaounde, Cameroon
[3]Department of Biochemistry, University of Douala, Cameroon

Abstract: The antihepatotoxic effects of *Xylopia phloiodora* extracts were evaluated in experimental models of liver injury in rats induced by CCl_4 or paracetamol. Crude extract (CE), ether extract (EE) and essential oils from stem bark or leaves were tested. Hepatic function was accessed by measuring serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in rats. Liver malondialdehyde (MDA) and reduced glutathione were also measured in control and treated rats. *X. phloiodora* leaves (CE) and stem bark (CE) extracts showed hepatoprotective activities at doses equivalent to 2.5 g of plant/kg, since serum levels of ALT and AST in rats given the extracts were significantly low ($p<0.05$ and $p<0.01$, respectively) when compared to control CCl_4-injured rats. Further studies were carried on the CE from stem bark of *X. phloiodora* which showed the highest level of protection against hepatitis. Further studies of the crude extract showed highest antihepatotoxic activity with the ether precipitate (PE) which was effective at 100mg/kg for hepatocurative activity in CCl_4-injured rats. In experiments comparing the PE (100 mg kg^{-1}) to a reference antihepatotoxic substance (silymarin) the PE exhibited a 71 and 80% hepatoprotection compared to the 80 and 90% one exhibited by silymarin in CCl_4-and paracetamol-injured rats respectively. This study demonstrated that ether precipitate of Xylopia phloiodora was effective in protecting the liver from toxic hepatitis.

Key words: Antihepatotoxic, *Xylopia phloiodora*, carbon tetrachloride, paracetamol, rat

INTRODUCTION

The *Xylopia* spp. (Annonaceae) are trees of 20 to 30 m height, found in the forest. Traditional beliefs from the Centre and South provinces of Cameroon attribute to *Xylopia* spp. their usefulness in the treatment of hepatitis and other liver ailments. Experimentally, most of the properties reported so far range from cytotoxic (Granell *et al*., 2004), cardiovascular and diuretic (Somova *et al*., 2001) and antimicrobial activities (Jenett-Siems *et al*., 1999; Tatsadjieu *et al*., 2003; Fekam *et al*., 2003) for *Xylopia aethiopica*, *columbiana* and *frutescens*. But no report so far has been done on the antihepatotoxic activity *Xylopia phloiodora* for which is traditionally claimed to heal hepatitis.

Carbon tetrachloride and paracetamol are known to cause liver damage (Recknagel, 1983; James *et al*., 2003). When administered to rats, they act by inducing oxidative damages to liver cells which leads to cellular necrosis, resulting in increases in serum enzymes SGOT and SGPT. These models of hepatotoxicity has been widely used to study the antihepatotoxic activities of exogenous drugs in experimental animal models (Shenoy *et al*., 2001; Bisshayi *et al*., 2002; James *et al*., 2003)

In view of confirming the alleged effects on hepatitis, various organic extracts from leaves and stem bark of *Xylopia phloiodora* were prepared and their antihepatotoxic effects evaluated in experimental models of liver injury in rats induced by CCl_4 or paracetamol.

MATERIALS AND METHODS

Animals: Male Wistar albino rats (Biochemistry Department Animal House, University of Yaounde I) weighing between 120-150 g were used. They were fed a standard laboratory diet (Laboratoire National Vétérinaire, Garoua, Cameroon) and given tap water *ad libitum* during all the experiments.

Corresponding Author: Paul F. Moundipa, Laboratory Biochemical Pharmacology and Toxicology, Department of Biochemistry, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon
Tel: +237 201 6043/731 0383 Fax: +237 222 13 20

Preparation of the plant extract: Stem bark and leaves of *Xylopia phloiodora* Mildbr. (X. *phloiodora)* were collected in may 2002 in the Centre province (Cameroon), air-dried and ground. The plants were extracted by maceration in a mixture of methylene chloride-methanol (1:1) for 48 h. The solvent was evaporated to dryness and the residue obtained used as crude extract (5% yield). The crude extract (100 g) was further treated with ether to yield 20 g of precipitate (PE) and 80 g of ether extract (EE) which was evaporated to dryness and used. Essential oils of *X. phloiodora* was also obtained from stem bark by hydro-distillation (0.3% yield).

Animal treatment: The animals were divided into normal, control and experimental groups (5 to 6 animals each). Hepatitis was induced in control and experimental groups of rats by intraperitoneal administration of CCl_4 (0.3 mL kg^{-1}) dissolved in corn oil (7 mL kg^{-1}). The normal group received only the vehicle. One hour before induction of hepatitis, crude extracts dissolved in DMSO (<10% v/v) were given orally to each experimental group of rats at a dose equivalent to 2.5 g of plant per kg of body weight (Fleurentin and Joyeux, 1990). The normal and control groups were given the vehicle. At the beginning of this study, an alkaloid rich fraction of *Enantia chlorantha* (BN95A) was used as positive control at a dose of 10 mg kg^{-1}, for its known antihepatotoxic activity (Virtanen *et al*., 1988). The animals were fasted but given water 12 h before blood collection. Half a mL of blood was collected from each rat by the jugular vein with a sterile syringe 24 h after CCl_4 administration. This period was chosen after preliminary trials to determine the appropriate period of blood collection following administration of the toxin. Serum was prepared from blood and used for biochemical analysis.

Antihepatotoxic studies of *X. phloiodora* extracts: Hepatitis was induced in male rats one hour before administration of the different extracts (CE, PE, EE and essential oils) from stem bark in order to assess the hepatocurative effects. The animals were treated as described above and doses given were computed on the same basis of equivalence to 2.5 g of plant material. Blood was collected from each animal for serum preparation and analysis.

A dose-response study was performed with PE. 50, 100 and 200 mg kg^{-1} extracts were given to rats according to groups and the animals treated as above. The controls received only the vehicle. Twenty four hours later, blood was collected from each rat for serum preparation and biochemical analysis.

Effect of PE on two models of liver injury: The antihepatotoxic effect of PE was also evaluated on CCl_4 and paracetamol-induced liver injury one hour after intoxication. Male rats were divided into 9 groups: 3 groups of normal rats and 6 groups of rats in which hepatitis was experimentally induced by CCl_4 or paracetamol (500 mg kg^{-1}) for 3 groups each. The control groups received the different vehicles of CCl_4, silymarin and paracetamol (corn oil, NaCl 0.9% and methyl cellulose 1%, respectively). Hepatitis was cured by administration of silymarin or PE at 100 mg kg^{-1}. Twenty four hours after hepatitis induction, the rats were killed by decapitation and blood collected for serum preparation. The liver was also removed after dissection, homogenised at 4°C in ice-cold KCl 150 mM (10% homogenate) and a post-mitochondrial supernatant prepared by centrifugation at 10,000 x g for 20 min at 4°C using a Beckman J2.21 model centrifuge. The serum and liver supernatants were used for biochemical analysis.

Biochemical analysis: The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) were measured by the method of Bergmeyer *et al*. (1978) and total bilirubin were assayed in the serum using Sigma kits (Sigma Chemical Co. MO St Louis). Liver proteins were estimated by the biuret method (Gornall *et al*., 1949) using bovine serum albumin as standard. Glutathione (Ellman, 1959) and malondialdehyde (MDA) (Wills, 1987) were also analysed in the liver.

Statistical analysis: All results were analysed by ANOVA and differences between means assessed by the Student t-test.

RESULTS

Serum ALT and AST levels in CCl_4-treated rats after treatment with leaf or stem bark extracts: As shown on Fig. 1 and 2, the serum levels of aminotransferase were significantly low when rats were given extracts from *X. phloiodora* after CCl_4 intoxication. For ALT the decrease was 52% for the extract from leaves of *X. phloiodora* ($p<0.05$) and 76% for stem bark extract ($P<0.001$) when compared to control group. The same pattern was observed with AST.

Serum ALT and AST levels in CCl_4-treated rats after treatment with stem bark extracts: Treatment of rats with PE resulted in a significant decrease of serum ALT and AST ($p<0.05$) when compared to the control (Fig. 3 and 4). The essential oils or the EE did not induced any change in the aminotransferase. For bilirubin its level was low in serum of rats treated with PE.

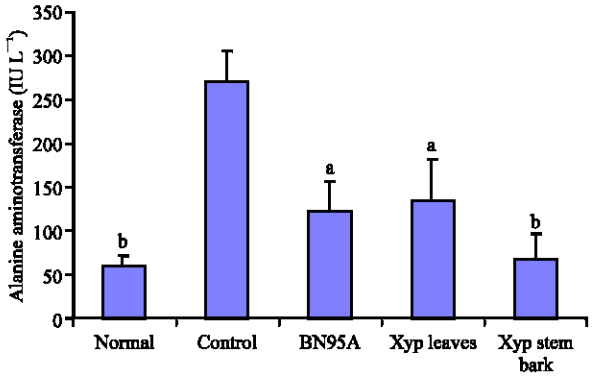

Fig. 1: Serum ALT in CCl_4-treated rats after treatment with plant extracts from *Xylopia phloiodora*. BN95A (alkaloid-rich fraction of *Enantia chlorantha*). Mean values of 5 to 6 rats significantly different from control at $^{a}p<0.05$ and $^{b}p<0.01$

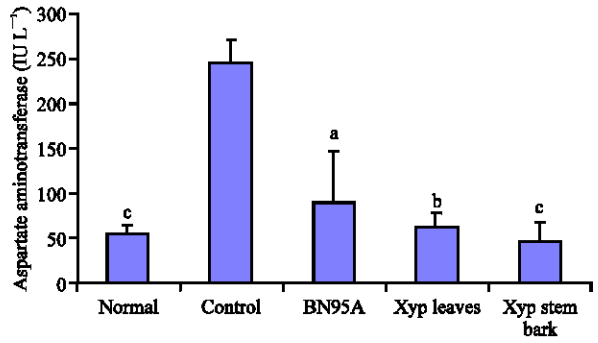

Fig. 2: Serum AST in CCl_4-intoxicated rats after treatment with plant extracts from *Xylopia phloiodora*. BN95A (alkaloid-rich fraction of *Enantia chlorantha*). Mean values of 5 to 6 rats significantly different from control at $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$

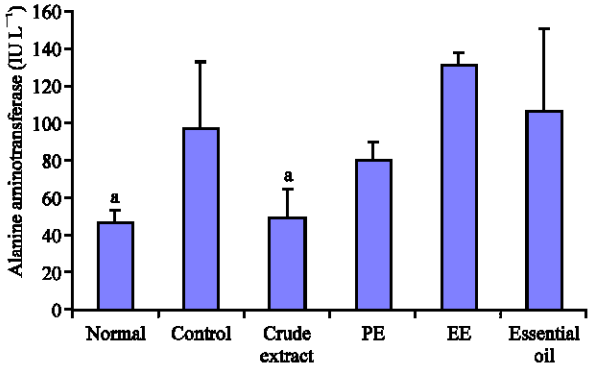

Fig. 3: Serum ALT in CCl_4-intoxicated rats after treatment with various stem bark extracts from *Xylopia phloiodora*. EE (ether extract), PE (ether precipitate). Mean values of 5 to 6 rats significantly different from control at $^{a}p<0.05$

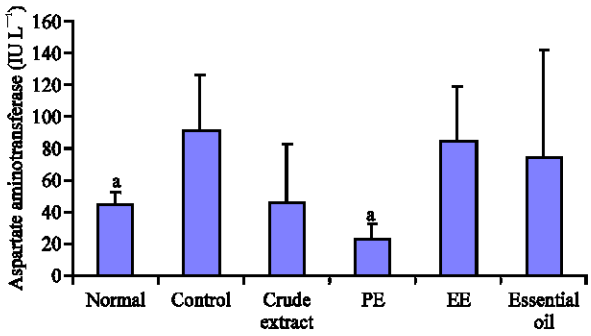

Fig. 4: Serum AST in CCl_4-intoxicated rats after treatment with various stem bark extracts from *Xylopia phloiodora*. EE (ether extract), PE (ether precipitate). Mean values of 5 to 6 rats significantly different from control at $^{a}p<0.05$

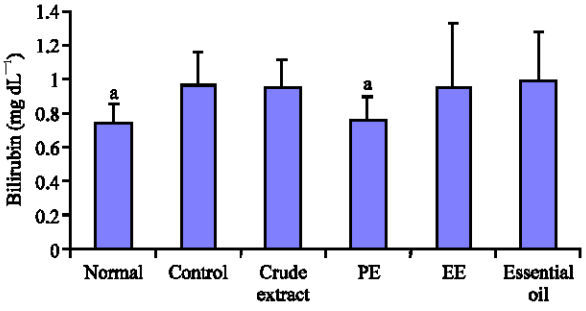

Fig. 5: Serum total bilirubin in CCl_4-intoxicated rats after treatment with various stem bark extracts from *Xylopia phloiodora*. EE (ether extract), PE (ether precipitate). Mean values of 5 to 6 rats significantly different from control at $^{a}p<0.05$

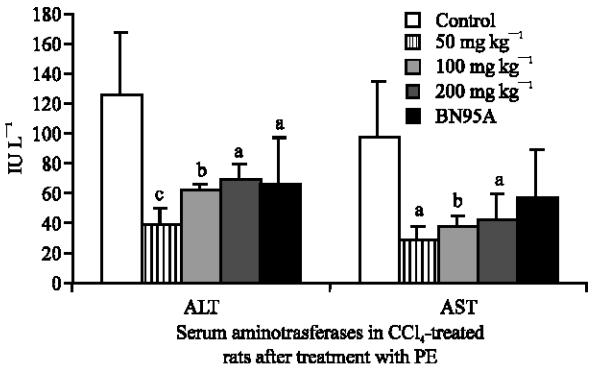

Fig. 6: Serum ALT and AST in CCl_4-intoxicated rats after treatment with PE (ether precipitate). Mean values of 5 to 6 rats significantly different from control at $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$

Table 1: Liver protein, glutathione and malondialdehyde in rats treated with CCl_4 after administration of PE (ether precipitate)

Groups	Protein level (mg g^{-1})	Glutathione level (µmole mg^{-1} protein)	Malondialdehyde level (nmole mg^{-1} protein)
CCl_4 vehicle	690.61±100.69^c	8.63±3.37	0.08±0.02^c
Silymarin vehicle	561.94±90.50^c	10.41±1.42	0.09±0.02^c
Control	201.31±11.20	6.23±2.20	0.33±0.14
PE (100 mg kg^{-1})	501.95±137.31^c	6.99±1.26	0.10±0.03^c
Silymarin (100 mg kg^{-1})	581.27±22.43^c	7.98±1.19	0.08±0.02^c

Values are mean ±SD. ^{c}Values significantly different from control group at $p<0.001$

Table 2: Liver protein, glutathione and malondialdehyde in rats treated with paracetamol after administration of PE (ether precipitate)

Groups	Protein level (mg g^{-1})	Glutathione level (µmole mg^{-1} protein)	Malondialdehyde level (nmole mg^{-1} protein)
Paracetamol vehicle	467.29±124.67^c	10.60±4.37	0.09±0.01^c
Silymarin vehicle	561.94±90.50^c	10.41±1.42	0.09±0.02^c
Control	271.97±36.78	7.52±1.10	0.23±0.05
PE (100 mg kg^{-1})	382.46±49.91^c	8.31±1.25	0.14±0.03^c
Silymarin (100 mg kg^{-1})	397.30±58.93^c	8.60±1.41	0.13±0.03^c

Values are mean±SD. ^{c}Values significantly different from control group at $p<0.001$

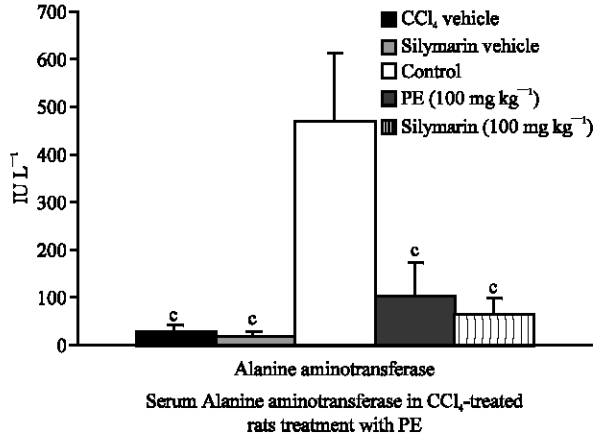

Fig. 7: Serum ALT in CCl_4-treated rats after treatment with PE (ether precipitate). Mean values of 5 to 6 rats significantly different from control at $^c p<0.001$

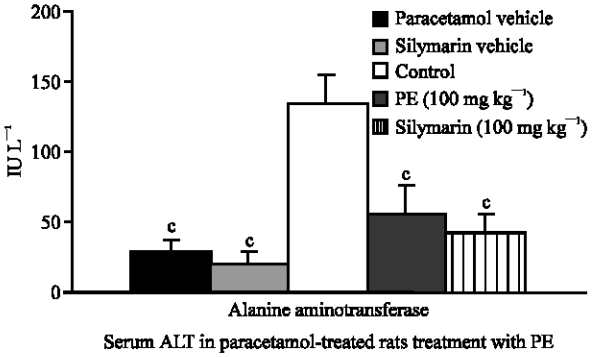

Fig. 8: Serum ALT in paracetamol-treated rats after treatment with PE (ether precipitate). Mean values of 5 to 6 rats significantly different from control at $^c p<0.001$

Effect of doses of PE on CCl_4-induced hepatitis: Administration of various doses of PE resulted in a significant decrease in rat serum aminotransferases after induction of hepatitis by CCl_4, when compared to control (Fig. 5).

Effect of PE from *X. phloiodora* on CCl_4- and paracetamol-induced liver injury: Treatment of rats with various vehicles did not alter serum ALT in normal rats. When liver injury was induced in rats, serum ALT levels were raised. Administration of either PE or silymarin led to a decrease in the aminotransferase in both types of liver injury ($p<0.001$) (Fig. 6, 7 and 8). The computed hepatoprotection was 81 and 71% for PE, 91 and 80% for silymarin in CCl_4- and paracetamol-induced liver injury respectively.

For the other biochemical parameters, mainly proteins and MDA their hepatic levels were maintained at their normal values in CCl_4-and paracetamol-treated rats upon administration of either PE or silymarin (Table 1 and 2).

DISCUSSION

Paracetamol-and CCl_4-induced hepatitis are usually used as experimental models in the search for new antihepatotoxic compounds (Fleurentin and Joyeux, 1990). Once introduced in the organism, CCl_4 is converted in the liver into a radical which reacts with molecular oxygen to form a trichloromethyl peroxyl radical. This compound attacks membrane polyunsaturated fatty acids and causes membrane lipid peroxydation (Recknagel, 1983) which leads to impairment of membrane function. When paracetamol is introduced in excess in the body, this compound is also metabolized in the liver to a reactive metabolite which reacts with enzymes and membrane components of liver cells which results in cellular lesion (Rang *et al*., 1999). In both cases, an increase in the serum of some liver enzymes such as ALT and AST is observed. An extract is said to be antihepatotoxic if it prevents the

increase in the level of these serum enzymes in animals in which hepatitis has been experimentally induced.

In present study, treatment of rats with CCl_4 led to an increase in serum aminotransferases and extracts from *X. phloiodora* exhibited hepatoprotective activity, since their administration led to a decrease in serum levels of ALT and AST, as well as BN95A, the used control. According to the percentage of decrease in the level of the aminotransferases it seemed that extracts from stem bark of *X. phloiodora* were more active than those from leaves. If we compare the doses used in this study to those found in literature (Prakash *et al*., 1995; Anand *et al*., 1994; Gonzalez *et al*., 1995) ours were generally low. This could mean that *X. phloiodora* extract has a high hepatoprotective potential. This potential is usually due to the presence in the extract of antioxydant constituents which scavenge reactive metabolites generated in the course of the metabolism of CCl_4 (Gonzales *et al*., 1995).

Further separation of the crude extract led us to obtain of PE, EE and essential oils. The study of these extracts revealed that the active components against hepatitis were found in the ether precipitate since this precipitate provoked significant reduction in the aminotransferases upon administration to CCl_4-treated rats. Moreover, this precipitate was active at doses of 50 and 100 mg kg^{-1} as shown by both ALT and AST levels in the serum as well as hepatic glutathione and MDA. This extract at a dose of 100 mg kg^{-1} was effective in protecting from liver injury in two models of experimental hepatitis as compared to a control antihepatitis reference compound, silymarin. This ether precipitate is the one to be used in further studies on the purification of the active components on *X. phloiodora* against hepatitis.

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