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Marie-Jeanne Mukazayire, V. Véronique Allaëys, Pedro Buc Calderon, Caroline Stévigny, Marie-Josée Bigendako, et al.. Evaluation of the hepatotoxic and hepatoprotective effect of Rwandese herbal drugs on (guinea pigs barbiturate-induced sleeping time) and (rat precision cut liver slices, PCLS) models. *Experimental and Toxicologic Pathology*, 2010, 62 (3), pp.289. 10.1016/j.etp.2009.04.005 . hal-00592285

HAL Id: hal-00592285

<https://hal.science/hal-00592285>

Submitted on 12 May 2011

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Author's Accepted Manuscript

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PII: S0940-2993(09)00171-7
DOI: doi:10.1016/j.etp.2009.04.005
Reference: ETP 50354

To appear in: *Experimental and Toxicologic Pathology*

Received date: 14 July 2008
Revised date: 30 January 2009
Accepted date: 22 April 2009

Cite this article as: Marie-Jeanne Mukazayire, Véronique Allaëys, Pedro Buc Calderon, Caroline Stévigny, Marie-Josée Bigendako and Pierre Duez, Evaluation of the hepatotoxic and hepatoprotective effect of Rwandese herbal drugs on *in vivo* (guinea pigs barbiturate-induced sleeping time) and *in vitro* (rat precision cut liver slices, PCLS) models, *Experimental and Toxicologic Pathology*, doi:10.1016/j.etp.2009.04.005

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**Evaluation of the hepatotoxic and hepatoprotective effect
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Abstract

Precision-cut liver slices (PCLS) preserve the tissular organization of the organ and represent an *in vitro* model closer to *in vivo* conditions than hepatocytes cultures. As this may be an interesting tool for the investigation of hepatotoxic and protective effects but also for bioguided fractionations schemes, the usefulness of PCLS was compared with an *in vivo* test of liver function. Crude extracts derived from five herbs used in Rwanda for hepatoprotective activity were tested on CCl₄-treated guinea pigs by the method of barbiturate-induced sleep modification. *Ocimum lamiifolium*, *Crassocephalum vitellinum*, *Guizotia scabra* and *Vernonia lasiopus* leaves extracts allowed animals to recover barbiturate sleep duration in proportions of 88, 78, 61 and 34 %, respectively and *Microglossa pyrifolia* was found inactive. Dried methanolic extracts of the 5 plants were then tested *in vitro* on rat PCLS for protection against acetaminophen - induced hepatotoxicity. In this model, *Guizotia scabra*, *Microglossa pyrifolia* and *Vernonia lasiopus* were found hepatotoxic by themselves and unable to prevent acetaminophen toxicity. The most active extract, obtained from *Ocimum lamiifolium*, was subjected to bioassay-guided fractionation by chromatography on Si-C18 to yield two quite active fractions.

From a single animal, at least 50 PCLS explants can be prepared, which allows testing a large amounts of samples, strengthening ethnopharmacological data on hepatoprotective medicinal plants and investigating hepatotoxic effects.

Key words: Rat precision-cut liver slices (PCLS); hepatotoxicity; hepatoprotection; *Ocimum lamiifolium*; *Crassocephalum vitellinum*; *Guizotia scabra*; *Vernonia lasiopus*; *Microglossa pyrifolia*; Guinea pigs; Sleeping time

1. Introduction

The liver, major organ of metabolism and excretion, is susceptible to a number of pathologies, classified as cirrhosis, acute chronic hepatitis and hepatitis; causes of hepatic trouble include parasitic and viral infections, autoimmune diseases and intoxication with various xenobiotics, such as chlorinated solvents, alcohol, drugs, herbal medicines, peroxidized fatty acids, fungal toxins, industrial pollutants and radioactive isotopes (Evans, 2002). The predominant pathologies in a given country depend on the lifestyle and economical conditions. In Rwanda, viral hepatitis and their complications, cirrhosis and hepatic carcinoma, represent 80 % of all liver pathologies, the 9th cause of morbidity (Musemakweli, 1999), and a number of plants are traditionally used to treat liver diseases (Van Puyvelde L., 1988). Excepting vaccines and interferon α -2b, which concern only viral infections, modern medicine is quite limited in preventing or treating hepatic diseases; the only drugs available are cholagogues, choleretics, drugs for cholesterolic lithiasis, N-acetyl-cysteine and flavo-lignanes obtained from *Silybum marianum*. This limitation of therapeutic options gives considerable interest to the search for active compounds from plants traditionally used for these affections (Evans, 2002). In this context, an ethnopharmacological inquiry of a series of herbalists and/or traditional healers for plants used as remedies for liver diseases was undertaken by our team in Butare, Rwanda, in 2004, and confronted with published documents from Rwanda (Van Puyvelde L. et al., 1977; Van Puyvelde L., 1988; Rwangabo, 1993) and other countries (Demissew and N., 1994; Neuwinger, 2000). This led to the selection of five herbs locally used for hepatoprotective activity (Table 1), *Crassocephalum vitellinum*, *Guizotia scabra*, *Microglossa pyrifolia*, *Ocimum lamiifolium* and *Vernonia lasiopus*. Such ethnopharmacological reports however do not give information enough regarding the clinical efficaciousness or safety of herbs and there are risks possibly associated with uncontrolled/unsubstantiated use of herbal remedies. The present study is

intended to explore whether these herbs could have protective effect on hepatocytes and to give an orientation to find hepatoprotective compounds that may be present in the extracts; this first step should eventually lead us to the molecules responsible for the effect to investigate mechanisms of action on the liver.

In order to investigate hepatotoxic and hepatoprotective compounds, i.e. agents capable to prevent and/or reverse the effects induced by an hepatotoxicant (CCl₄, D-galactosamine, acetaminophen, peroxides,...), a series of models have been developed, either *in vivo* (histology, measurement of serum hepatic enzymes, barbiturate-induced sleeping time, prothrombine time, bromosulphaleine clearance) or *in vitro* (continuous cell lines, primary cultures of hepatocytes). Precision-cut liver slices (PCLS) preserve the tissular organization of the organ and represent an *in vitro* model closer to *in vivo* conditions than hepatocytes cultures (Morales et al., 1998; Vanhulle et al., 2001; Vickers and Fisher, 2004). As PCLS may be an interesting tool for the investigation of hepatotoxic and protective effects but also for bioguided fractionation schemes, their usefulness has been further investigated on the five ethnopharmacologically selected Rwandan herbal drugs, comparing to an *in vivo* test of liver function, barbiturate-induced sleeping time.

2. Material and methods:

2.1. Plant material

The leaves of *Crassocephalum vitellinum* (Benth) S. Moore (Asteraceae), *Guizotia scabra* (Vis) Chiov (Asteraceae), *Microglossa pyrifolia* (Lam.) Kuntze (Asteraceae), *Ocimum lamiifolium* Hochst ex. Benth (Lamiaceae) and *Vernonia lasiopus* O. Hoffm (Asteraceae) were collected in the prefecture of Butare (South-Western Rwanda) in July 2004, air-dried in the shade and mechanically powdered. The plants were authenticated by Dr M.J. Bigendako, Center of Research in Phytomedicals and Life Sciences, Butare, Rwanda and voucher specimens were deposited in the BRLU herbarium, Belgium.

2.2. Chemicals

Williams' medium E (WME) and foetal calf serum (FCS) were purchased from Gibco BRL (Middlesex, UK), acetaminophen from Janssen Pharmaceutica (Beerse, Belgium), insulin (Actrapid HM) from Novo Nordisk (Bagsvaerd, Denmark), gentamicine sulfate, carbon tetrachloride, pentobarbital, N-acetyl cysteine (NAC), β -glucuronidase, benzoxazolinone chlorzoxazone and 6-OH- chlorzoxazone from Sigma-Aldrich (Bornem, Belgium).

2.3. Extraction of plant material

2.3.1. Extracts for *in vitro* tests and phytochemical screening

Each plant (50 g) was macerated for 24 h and exhaustively percolated with solvents of increasing polarities, N-hexane, chloroform, methanol and water. The resulting extracts were evaporated to dryness (reduced pressure, 40°C), yielding four crude extracts (Table 2) which were subjected to phytochemical analysis, the methanolic extract being also used for *in vitro* tests.

500 mg of the most active methanolic extract (*Ocimum lamiifolium*) were submitted to column chromatography on silica gel-C18 eluting with a gradient of water - methanol (100% water, 90:10, 80:20, 60:40, 50:50 and 100% methanol); 60 fractions of 10 ml were collected and combined upon thin-layer chromatography (TLC) analysis to yield 9 fractions (A to I).

2.3.2. Extracts for *in vivo* tests

Crude extracts were prepared for the 5 plants by a healer working in the dispensary of the Institute of Scientific and Technological Research of Butare, Rwanda, following his traditional protocol. Briefly, 350 g of each plant were infused in about 2000 ml of tap water,

filtered on a cotton cloth and dried under reduced pressure at 40°C to yield the extracts.

The traditional use of the five plants relying on aqueous decoctions, only the polar extracts (aqueous decoction and methanolic leaves extracts) were selected for pharmacological testing.

2.4. Animals

Male Wistar rats, weighing 250 - 300 g, were purchased from Iffa-credo (Les Oncins, France), housed in individual cages in a temperature and light controlled room (12 h dark/light cycles) receiving a standard diet AO3 (U.A.R., Villemoisson sur Orge, France) and water ad lib.

Male and female healthy guinea pigs, weighing 500 - 600 g and raised in the Institute for Research, Science and Technology (IRST, Huye, Rwanda). Before the experiment, all guinea pigs were fasted for 16 hours and, throughout the duration of *in vivo* experiments (3 days), they were fed with a thorough mixture of 2 edible leaves, *Lactuca capensis* (Thunb.) (Asteraceae) and *Brassica oleracea* (var. *capitata*) (Brassicaceae) and tap water. These plants are known to be edible vegetables; our experience shows that guinea pigs can be grown with this regimen without any apparent problem. As control animals were fed the same mixture, diet parameters should be under control. All experiments were conducted in accordance with international standards of animal welfare as recommended by the European Union on Animal Care (CCE Council 86/609).

2.5. Measurement of hepatoprotective activity

2.5.1. *In vitro* tests on acetaminophen-treated precision-cut liver slices (PCLS)

Surgical procedures were carried out on fed rats under pentobarbital (60 mg/kg) anaesthesia. The liver was perfused *in situ* with ice-cold Krebs-Ringer solution before slicing. PCLS (250 - 300 µm thickness) were prepared from the whole liver without distinction of lobes in oxygenated ice-cold Krebs-Ringer buffer by using a Krumdieck tissue slicer according to procedures previously described (Evdokimova et al., 2001). They were stored 30 min at 4°C in WME containing FCS (10% v/v), glutamine (2 mM), insulin (100 nM) and dexamethasone (10 nM) and then transferred to 25-ml vials containing WME (2 slices/4 ml) supplemented with glutamine (2 mM), insulin (100 nM) and gentamicin sulfate (50 µg/ml). PCLS were incubated in a shaking water-bath (100 cycles/min) at 37°C under a continuous flow of O₂ -

CO₂ (95 % : 5%) and randomized to avoid any variability between slices that may come from localization in the liver lobes or size. After 1 hour preincubation, allowing fresh slices to recover ATP content, the slices were transferred to other vials containing fresh medium and supplemented with different combinations of acetaminophen (hepatotoxic agent, 10 mM), N-acetyl cystein (hepatoprotective agent, 20 mM) and / or plant extracts (1 mg/ml) for 24 h. Unsupplemented control slices were sampled after this 24 h incubation (Control). As the PCLS test is intended for bioguided fractionation (*i.e. the use of a bioassay to guide the purification and isolation of active compounds*), and given the relatively low amounts of material to be purified, a preliminary study led us to select a sensibly low dosage for tested extracts (1 to 5 mg/ml); this is the same order of magnitude as acetaminophen (1.5 mg/ml) and NAC (3.3 mg/ml).

Liver slices were taken, washed twice in saline and sonicated immediately in 1 ml of 2 % perchloric acid. The intracellular ATP content was measured on neutralized perchloric acid extracts using the ATP Bioluminescence Assay Kit CLS II from Boehringer-Mannheim (Germany). Protein measurement was performed in duplicate on sonicated PCLS by the method of Lowry et al, 1951 using bovine serum albumin as standard (Lowry et al., 1951). For each incubation condition, 2 slices were tested with 2 ATP measurements per slice; samples were compared by ANOVA and pairwise comparisons were performed by a *t-test* with Bonferroni correction. For histological examination, control and treated PCLS were immersed in 10 % formaldehyde, embedded in paraffin, sliced with a microtome, layered on microscope slides and stained with haematoxyline-eosine. CYP2E1 activity was measured by incubation of PCLS with chlorzoxazone and HPLC measurement of its hydroxylated metabolite, 6-OH-chlorzoxazone (Wauthier et al., 2004). Briefly, 4 slices per experimental condition in 2 ml medium were added with 20 µl of 10 mg/ml chlorzoxazone in methanol and incubated (37°C, 90 min); 300 µl of medium were added with 1.2 IU β7

glucuronidase in 100 µl acetate buffer (0.3 M, pH 5) and further incubated (37°C, 2h). Upon addition of 150 µl ZnSO₄ (15 % in water) and 100 µl benzoxazolinone (0.1 mg/ml in methanol, internal standard) and centrifugation (13000 g, 1 min), the supernatant was filtered (PVDF 4mm, 0.45µm) and analyzed by HPLC in the following conditions : column C18 BDS 3 µm (LC Packings); mobile phase, ammonium acetate (3.9 g/l, pH 4.25) - acetonitrile (84:16) at 1.5 ml/min; UV detection at 287 nm.

2.5.2. In vivo tests on CCl₄-treated male and female guinea pigs

Guinea pigs were divided into groups of 8 animals each, including a control group (no treatment prior to determination of sleeping-time). Animals were inspected every 12 h for humane endpoints, including behaviour (abnormal movements, immobility), clinical signs (swelled abdomen, signs of severe distress, diarrhoea, convulsions) and eventual signs of impending death (convulsions, lateral position, recumbence, tremor); none of the animals had to be withdrawn from the study during the 3-days experiments. At day-1, the CCl₄ group was given orally 1.5 mg/kg carbon tetrachloride as a fresh mixture with an equal volume of peanut oil. The duration of the sleep was determined two days after the administration of carbon tetrachloride (day-3). In this model, pure compounds have previously been tested from 200 to 800 mg/kg (Van Puyvelde L. et al., 1989); reasoning that the extracts are most probably mixtures of active and inactive compounds, with unknown bioavailability, we selected a i.p. dose of 1 g/kg. At day-1, the treated groups received the preparation orally in doses of 1 g extract/kg one hour prior to carbon tetrachloride. The second and third day, the same dosage of the preparation was given and the duration of sleep determined on the third day, thirty minutes after the administration of the last preparation. Animals received pentobarbital intraperitoneally (i.p.) in doses of 50 mg/kg (the barbiturate was dissolved in 0.1 N NaOH). For each animal, sleep duration was defined as the time between loss and recovery of righting reflex.

2.6. Preliminary phytochemical analysis

Phytochemical screening of the plants was undertaken using described methods (Rwangabo, 1986; Wagner and Bladt, 1996). The screening covered (i) alkaloids (Mayer and Dragendorff's reagents; silica gel TLC in toluene-ethyl acetate-diethylamine (70:20:10) with spraying by Dragendorff and iodoplatinate reagents); (ii) terpenoids -sterols (Liebermann's reagent; silica gel TLC in toluene-chloroform-ethanol (40:40:10) with spraying by anisaldehyde-sulphuric acid; (iii) flavonoids (Shinoda's reagent); (iv) anthocyanins (HCl 2N); (v) saponins (honeycomb froth persisting for at least 30 min after vigorous shaking of the water extract; Liebermann-Buchard's test); (vi) tannins (ferric chloride reagent and gelatin-salt block test); and (vii) anthraquinones (Borntrager's reagent).

3. Results

Crassocephalum vitellinum, *Ocimum lamiifolium*, *Guizotia scabra*, *Microglossa pyrifolia*, and *Vernonia lasiopus* were selected according to an ethnopharmacological inquiry and their use in liver diseases (Table 1). The leaves of the five plants were extracted with different solvents and their extraction yields are presented in Table 2. Each plant was screened for the main classes of phytochemicals (Table 3), revealing the presence of alkaloids, flavonoids, terpenoids - steroids, saponins and tannins as major chemical constituents.

Figures 1 and 2 compare the effects of the five different methanolic extracts with NAC as reference hepatoprotective agent in acetaminophen-challenged PCLS incubated in Williams' medium either with or without paracetamol. On PCLS *Guizotia scabra*, *Microglossa pyrifolia* and *Vernonia lasiopus* were found hepatotoxic by themselves (reduction in ATP levels, 68.7 %, 89.7 % and 88.3 %, respectively) and unable to prevent acetaminophen toxicity (Figure 1).

Crassocephalum vitellinum and *Ocimum lamiifolium* were not hepatotoxic and protected the liver slices against the induced hepatotoxicity (Figures 1 and 2). NAC proved extremely

efficient in preventing acetaminophen damage; indeed NAC forms a nucleophilic adduct with the electrophilic toxic quinoneimine metabolite (NAPQI). The *Crassocephalum vitellinum* and *Ocimum lamiifolium* extracts were comparatively less efficient than NAC, which may come from a different mechanism of action or a lower dose of active compounds in the tested extracts. Fractionation of the *Ocimum lamiifolium* by column chromatography on C18-modified silica gel and elution by a gradient water : methanol yielded 9 subfractions, Ocimum A to I that present different TLC profiles. These were similarly tested on PCLS (data not shown) but only the first 2 fractions were found to prevent acetaminophen toxicity (Figure 2).

The experiment was repeated on a series of animals for the extracts yielding a positive response (Table 4). Histological examination of slices (Figure 3) shows that acetaminophen induces altered and necrotic cells with widened sinusoidal spaces and chromatolytic nuclei. NAC, *Crassocephalum vitellinum* and *Ocimum lamiifolium* extracts protect the tissue against acetaminophen toxicity, nuclei being apparent on a dense and coloured cytoplasm with normal sinusoidal spaces. Acetaminophen toxic metabolite, N-acetyl-p-benzoquinoneimine, is mainly produced by the cytochrome CYP2E1; the influence of active extracts on CYP2E1 was investigated by measuring the metabolism of a tracer compound, chlorzoxazone (Table 5). Whereas *O. lamiifolium* fraction A had no effect by itself on control slices, the *C. vitellinum* methanolic extract slightly reduced CYP2E1 activity, which may partly account for its protective activity. The formation of 6-OH-chlorzoxazone was severely decreased by acetaminophen treatment (3 % vs control), which could partly be prevented by *C. vitellinum* methanolic extract (40 %) and by *O. lamiifolium* fraction A (51 %). These preliminary data however show no dose-effect relationship and need further investigation.

In *in vivo* studies, liver injury induced by CCl₄, the best-characterized system of xenobiotic-induced hepatotoxicity, is a commonly used model for screening the anti-hepatotoxic / hepatoprotective activity of drugs (Brattin et al., 1985; Williams and Burk, 1990; Recknagel,

1991). As pentobarbital is metabolised exclusively in the liver, the sleeping time after a given dose is a measurement of hepatic metabolism. After poisoning with carbon tetrachloride, liver damage causes a sleeping time increase (Table 6); in the presence of an hepatoprotective agent, sleeping time is wholly or partly restored (Wagner and Wolff, 1977). The *Microglossa pyrifolia* aqueous extract does not protect the animals from CCl₄ toxicity; protection afforded by *Vernonia lasiopus* was extremely modest (34.1 %). *Guizotia scabra*, *Crassocephalum vitellinum* and *Ocimum lamiifolium* allowed recovering sleeping time by 61.4 %, 77.9 % and 88.2 %, respectively. These data partly confirm those obtained on PCLS with methanolic extracts in which *Guizotia scabra*, *Microglossa pyrifolia* and *Vernonia lasiopus* were found hepatotoxic by themselves, whereas *Crassocephalum vitellinum* and *Ocimum lamiifolium* protected the liver slices against the acetaminophen hepatotoxicity..

4. Discussion

By contrast to many other pathologies, the symptomatology of a number of hepatic troubles (icterus) is evident for traditional healers who can easily evaluate the response to treatments and thus **probably** select efficient herbal medicines. Many studies have already led to the characterization of more than 170 constituents isolated from 110 plants belonging to 55 families, including terpenoids, curcuminoids, lignoids and flavonoids and cyanogenetic glycosides (Luper, 1998; 1999; Evans, 2002; Seef et al., 2002; Thyagarajan et al., 2002) and a huge number of plants from India, China and Europe have been reported to treat liver diseases and boost liver functions (*Curcuma longa*, *Picrorrhiza kurroa*, *Camellia sinensis*, *Glycyrrhiza glabra*, *Bupleurum falcatum*, *Silybum marianum*, *Taraxacum officinale*,...) (Kiso et al., 1984; Sugiyama et al., 1998; Ram, 2001; Evans, 2002; Thyagarajan et al., 2002), including some species related to those of the present study, *Ocimum gratissimum*, *Ocimum sanctum* and *Crassocephalum crepidioides* (Chattopadhyay et al., 1992; Effraim et al., 2002; Aniya et al., 2005). By contrast, only relatively scarce data are available on the African traditional

pharmacopoeia and the five plants we selected for this study, according to an ethnobotanical enquiry, have not been previously investigated for hepatoprotective activity.

Experimentally the concept of liver protection strongly depends on the chosen toxicant and laboratory animal species, which may lead to the selection of plant extracts and compounds active on a given model but without clinical relevance. To maximize the chances to select active extracts, two biological models, *in vitro* tests on acetaminophen-treated rat liver slices and *in vivo* tests on carbon tetrachloride-challenged guinea pigs, were selected for their different toxicity mechanistic. CCl₄ is metabolised by CYP2E1 in an extremely reactive radical, CCl₃[•] that peroxidizes lipids, inducing hepatic lesions (Plaa and Hewitt, 1998). Acetaminophen (paracetamol), a frequently used analgesic and antipyretic drug, is primarily metabolized by sulfation and glucuronidation to unreactive metabolites, whereas a minor but highly reactive N-acetyl-*p*-benzoquinoneimine is normally detoxified by glutathione. Following overdosage, the glutathione pool gets depleted and this metabolite accumulates to produce liver injury (Kupeli et al., 2006). *In vitro* studies on acetaminophen-treated PCLS show that N-acetylcysteine (NAC), a major antidote in clinical cases of acetaminophen intoxications, prevents depletion of ATP; NAC also offers an hepatoprotective effect against other toxins known to deplete cellular ATP levels (Popat et al., 2002).

As both animal species investigated (rat and guinea pig) have different sensitivity to toxic action of chemicals and significant differences in xenobiotics metabolism, this widens the research for possible hepatoprotective mechanisms and compounds; the ultimate challenges remain however (i) the translation from hepatoprotection to a potential beneficial effect on chronic diseases such as viral hepatitis or cirrhosis, which is still quite undocumented; and (ii) the validation of such data on humans. On our 2 models, the *Ocimum lamiifolium* and *Crassocephalum vitellinum* polar extracts were found particularly active by contrast with *Microglossa pyrifolia* (no activity on both models) and *Vernonia lasiopus* (modest activity

only on the *in vivo* model). A discrepancy was observed between models for the extracts of *Guizotia scabra*; this may come from differences in toxicant activity mechanism, in the composition of tested extracts and/or doses.

The *O. lamiifolium* methanolic extract is already known for antipyretic activities in mice (Makonnen et al., 2003) and for the presence of a major flavonol O-glycoside, the quercetin 3-O-xyloseyl galactoside, and large amounts of flavone 5-O-glycosides (Grayer et al., 2002). *Crassocephalum vitellinum* and *Guizotia scabra* proved active on ileon and uterus of guinea pigs and on the arterial pressure of rabbits (Chagnon, 1984). Sesquiterpene lactones and other terpenoids were reported from these two herbs (Zdero et al., 1991; Zollo et al., 2000). *Vernonia lasiopus* is known for antiulcerous and analgesic activities (Johri et al., 1995). Some glucosides with antibacterial activity and antimalaric terpenoids have been isolated from *Microglossa pyrifolia* (Rucker et al., 1994; Köhler et al., 2002).

The hepatoprotective ability of *O.lamiifolium* and *C.vitellinum* polar extracts may be connected with their flavonoids, since these phytochemical constituents, detected in our active extracts, have been implicated as the hepatoprotective factors in *Crassocephalum crepidioides* (Asteraceae) on CCl₄-induced toxicity (Aniya et al., 2005) and in *Equisetum arvense* L. (Equisetaceae) on tacrine-induced toxicity in Hep G2 cells (Hyuncheol, 2004).

Further work is in progress to determine if any of these compounds may be responsible for the hepatoprotective activities of *Crassocephalum vitellinum* and *Ocimum lamiifolium*.

To our best knowledge, this is the first time that the PCLS model is proposed and used for the bioguided fractionation of active extracts. PCLS indeed represent an attractive model in the study of natural products, a fair number of tests being carried on the explants of a single animal. The PCLS however present the drawback of not being perfused, the liver is simply bathing in the nutrient medium. Compared to *in vivo* conditions, in which the liver is continually perfused by blood circulation, sensibly higher concentrations of test compounds

consequently have to be used. Compared to *in vitro* tests on monolayer cell cultures, higher concentrations of tested compounds are also required as cells inside the tissue need the diffusion of compounds through external cell layers (including external layers of dead cells induced by the slicing process). Despite this shortcoming, the PCLS model allows strengthening ethnopharmacological data on hepatoprotective medicinal plants and investigating hepatotoxic effects while sensibly reducing the number of experimental animals. Our results support the Rwandese traditional use of four of the five plants as antihepatitis remedies; they suggest *in vivo* hepatoprotective effects of *Ocimum lamiifolium*, *Crassocephalum vitellinum*, *Guizotia scabra* and *Vernonia lasiopus* aqueous extracts. Given the direct toxicity of *Guizotia scabra*, *Microglossa pyrifolia* and *Vernonia lasiopus* methanolic extracts on the liver slices model, these 3 herbs should be further evaluated; their eventual use in traditional medicine should be quite cautious and accompanied by a monitoring of the hepatic function. It is noteworthy that the 2 herbs that proved active and non toxic on our models, *C. vitellinum* and *O. lamiifolium* leaves, are edible vegetables or food additives in Tanzania (Copeland, 2004) and Ethiopia (Demissew and N., 1994), respectively; this gives some more indications of a probably safe medicinal use. Further studies are worthy being carried out on the active compounds of *Ocimum lamiifolium* and *Crassocephalum vitellinum* in order to assess their structure and elucidate their mechanism of action.

Acknowledgements

The authors are extremely thankful to the Belgium Technical cooperation (BTC), the Belgian Fonds National de la Recherche Scientifique and the Institute of Research, Science and Technology (IRST) of Rwanda for financial support of this work. The technical help of M. Faes and O. Vaillant is gratefully acknowledged.

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Accepted manuscript

Legends to the figures

Figure 1

Effect of N-acetylcysteine (20 mM) and the methanolic extracts of *Ocimum lamiifolium*, *Guizotia scabra*, *Microglossa pyrifolia* and *Vernonia lasiopus* (1 mg/ml) on acetaminophen challenged (10 mM) and control precision-cut liver slices.

Mean + SD of duplicate ATP measurements on 2 PCLS obtained from the same rat;

*** : $p < 0.001$, NS : $p > 0.05$ (ANOVA with post-hoc pairwise comparisons; *t*-test with Bonferroni correction).

Figure 2

Effect of N-acetylcysteine (20 mM), of the *Ocimum lamiifolium* and *Crassocephalum vitellinum* leaves methanolic extracts and of 2 fractions obtained from the *O. lamiifolium* extract (1 mg/ml) on acetaminophen-challenged (10 mM) and control precision-cut liver slices.

Mean + SD of duplicate ATP measurements on 2 PCLS obtained from the same rat;

*** : $p < 0.001$, NS : $p > 0.05$ (ANOVA with post-hoc pairwise comparisons; *t*-test with Bonferroni correction).

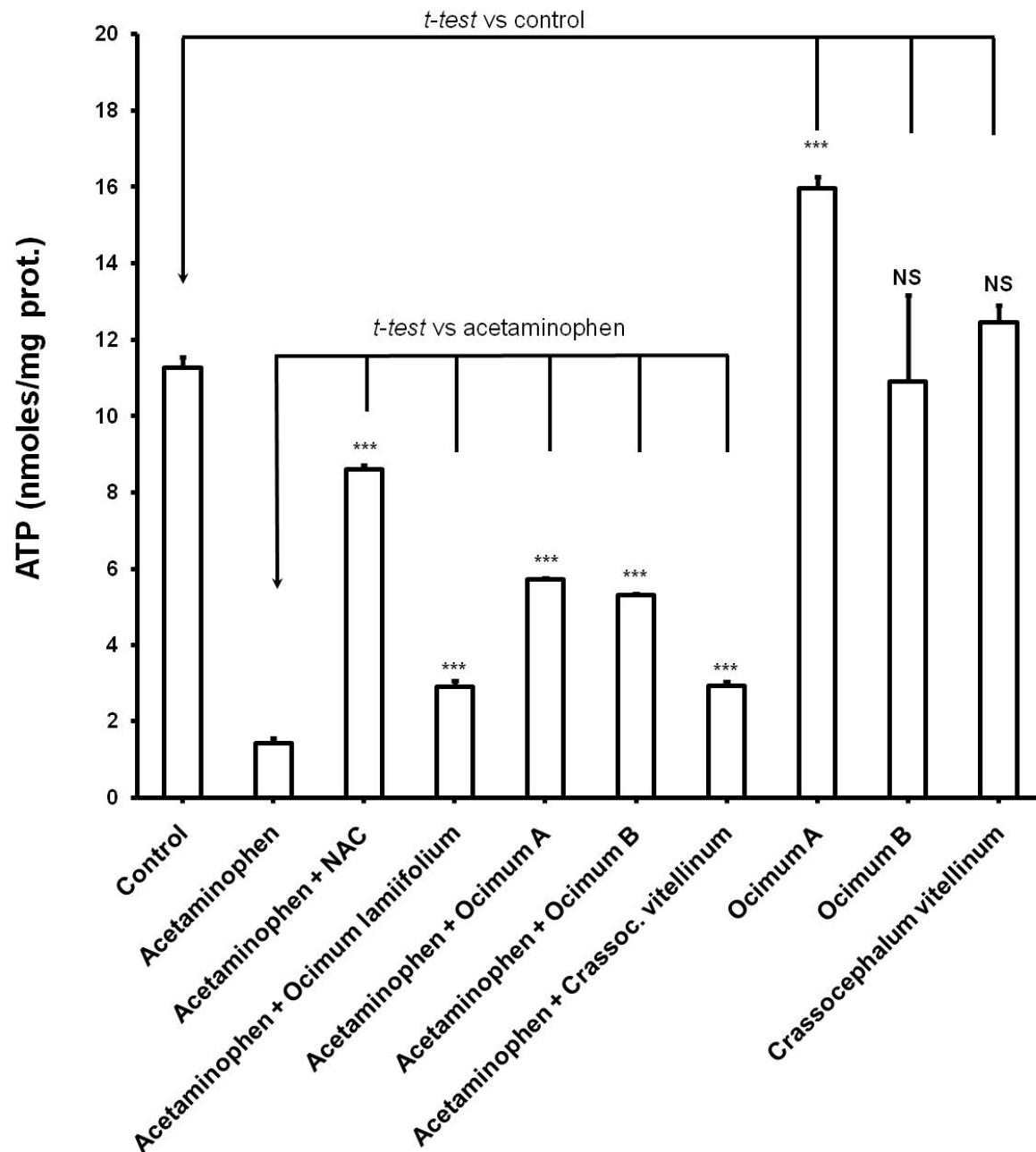
Figure 3

Effect of N-acetylcysteine (20 mM), of the *Ocimum lamiifolium* and *Crassocephalum vitellinum* leaves methanolic extracts on acetaminophen-challenged (10 mM) PCLS. (haematoxyline-eosine staining).

The bar chart displays ATP levels (nmoles/mg prot.) for various treatments. The y-axis ranges from 0 to 8. The x-axis lists the following treatments: Control, Acetaminophen, Acetaminophen + Nac, Acetaminophen + Guizotia scabra, Acetaminophen + Ocimum lamiifolium, Acetaminophen + Microgl. pyrif., Acetaminophen + Vernonia lasiopos, Guizotia scabra, Ocimum lamiifolium, Microglossa pyrifolia, and Vernonia lasiopos.

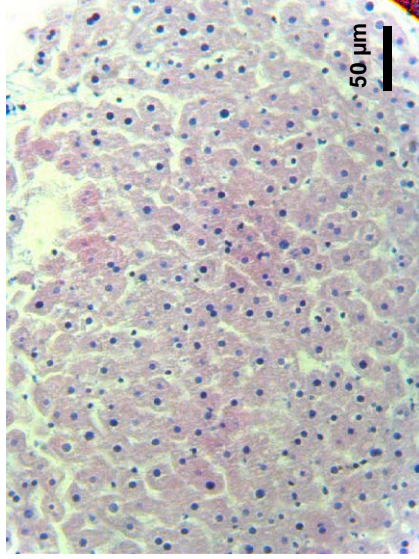
Significance levels are indicated by asterisks (***) and NS (Not Significant). Brackets indicate comparisons between groups.

Treatment	ATP Level (nmoles/mg prot.)	Significance vs Control	Significance vs Acetaminophen
Control	~6.9	-	-
Acetaminophen	~0.7	**	-
Acetaminophen + Nac	~3.8	**	***
Acetaminophen + Guizotia scabra	~0.6	**	NS
Acetaminophen + Ocimum lamiifolium	~1.1	**	***
Acetaminophen + Microgl. pyrif.	~0.6	**	NS
Acetaminophen + Vernonia lasiopos	~0.7	**	NS
Guizotia scabra	~2.1	**	***
Ocimum lamiifolium	~6.3	**	NS
Microglossa pyrifolia	~0.7	**	***
Vernonia lasiopos	~0.8	**	***

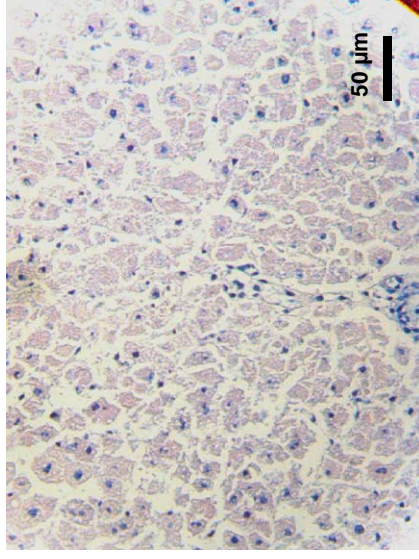
Figure 2 :

Mukazayire - Figure 2

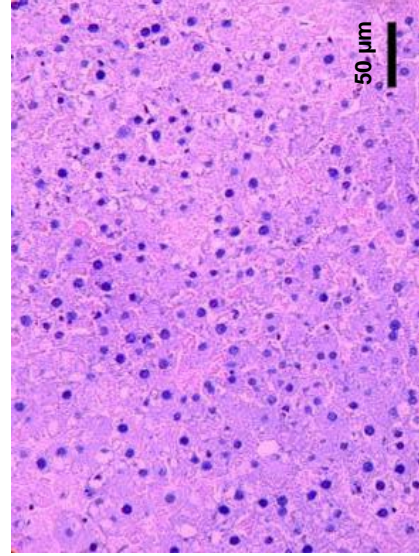
Control (24 h)



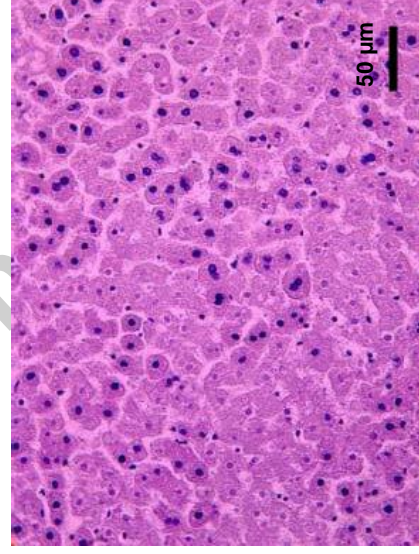
Acetaminophen (10 mM)



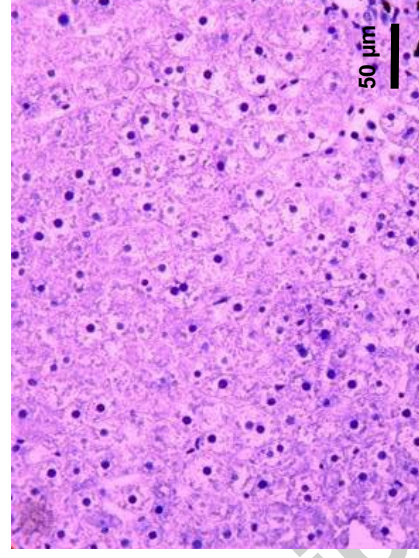
Acetaminophen (10 mM)
+ NAC (20 mM)



Acetaminophen (10 mM)
+ *O. lamifolium* methanolic extract (2 mg/ml)



Acetaminophen (10 mM)
+ *C. vitellinum* methanolic extract (2 mg/ml)



Mukazayire - Figure 3

Table

Table 1: Traditional uses of the herbs investigated.

Scientific name (family; organ)	Local name	Traditional uses ^(a)
<i>Ocimum lamiiifolium</i> Hochst ex. Benth (Lamiaceae; leaves)	Umusura	Hepatitis, gonorrhea, gastric ulcer asthma, dropsy
<i>Crassocephalum vitellinum</i> (Benth) S.Moore (Asteraceae; leaves)	Isununu	Hepatitis, woman sterility, strong fever, dysmenorrhea, facilitates the deliverance of placenta, constipation and kids' diseases
<i>Guizotia scabra</i> (Vis)Chiov (Asteraceae; leaves)	Ishikashike	Hepatitis, malaria, against helminthes
<i>Vernonia lasiopus</i> O.Hoffm (Asteraceae; leaves)	Ivumo	Hepatitis, wounds and kid's constipation
<i>Microglossa pyriformis</i> (Lam.) Kuntze (Asteraceae; leaves)	Umuhe	Hepatitis, malaria, rheumatism, gastric ulcer, wounds disinfection, eyes treatment, head and abdomen pain

^(a) Data combined from the present work and from Van Puyvelde, 1988

Table 2 : Extraction yields (m/m) for the 5 plants selected
(50 g leaves + 500 ml solvent; sequential 24 h macerations with increasing polarity solvents)

Solvent	Yield (% m/m)				
	<i>Crassocephalum vitellinum</i>	<i>Guizotia scabra</i>	<i>Microglossa pyrifolia</i>	<i>Ocimum lamiifolium</i>	<i>Vernonia lasiopus</i>
n-Hexane	1.4	1.6	2.5	3.2	7.4
Chloroform	1.6	4.9	5	3.2	41.1
Methanol	9	13.9	18	6.4	8.4
Water	8.6	11.9	15.9	21.7	7.8

Table 3 : Results of the phytochemical screening

Plant	Alkaloids		Terpenoids and steroids				Flavonoids				Anthocyanins		Saponins ^(b)		Tannins		Anthraquinones	
	P ^(a)	C	P	H	C	M	W	P	H	C	M	W	P	W	P		P	
<i>Crassocephalum vittelinum</i>	+/-	+/-	+	+	+	+	-	+	+	-	+	+	+	(2.2cm)	+	+	-	
<i>Guizotia scabra</i>	-	-	+	+	+	-	-	+	+	-	+	+	+	(1cm)	+	+	+	
<i>Microglossa pyrifolia</i>	+	+	+	+	+	+	-	+	+	-	-	+	+	(1.7cm)	+	+	-	
<i>Ocimum lamiifolium</i>	+	+	+	+	+	+	+	+	+	-	+	-	+	(2cm)	+	+	-	
<i>Vernonia lasiopus</i>	+	+	+	+	+	+	-	+	+	-	-	+	+	(1.5cm)	+	+	-	

^(a) Tested material (P = powder, H = N-hexane extract, C = chloroform extract, M = methanol extract, W = aqueous extract)

^(b) In brackets, height of foam column

^(c) Shinoda's reagent : red coloration (flavonols)

^(d) Shinoda's reagent : orange coloration (flavones)

^(e) reagent : violet coloration (flavonones)

Table 4 : Effect of N-acetylcysteine (20 mM) and the methanolic extracts of *Ocimum lamiifolium* and *Crassocephalum vitellinum* (1 mg/ml) on acetaminophen-challenged (10 mM) precision-cut liver slices from different animals

	Number of animals ^(a)	ATP (nmoles/mg proteins)		
		Mean ^(b)	SD	RSD (%)
Control	25	6.9 ^(***)	2.7	39 %
Acetaminophen	24	1.5	1.0	64 %
Acetaminophen + NAC	12	6.4 ^(***)	2.5	39 %
Acetaminophen + <i>Crassocephalum vitellinum</i>	6	3.2 ^(*)	0.4	13 %
Acetaminophen + <i>Ocimum lamiifolium</i>	10	4.5 ^(***)	1.4	31 %
Acetaminophen + <i>Ocimum lamiifolium</i> fraction A	4	8.2 ^(***)	5.1	63 %

(a) 2 slices per animal

(b) Kruskal-Wallis post-hoc pairwise comparisons versus treatment Acetaminophen (Bonferroni correction; ***, $p < 0.001$; *, $p < 0.05$)

Table 5 : CYP2E1 activity of acetaminophen-challenged (10 mM) and control precision-cut liver slices upon treatment with N-acetylcysteine, *Crassocephalum vitellinum* (methanolic extract) and *Ocimum lamiifolium* (fraction A)
(triplicate measurements on 4 PCLS obtained from the same rat; RSD = 6.2 %)

Treatment	6-OH-chlorzoxazone	
	µg/mg prot	% vs control
Control (24h incubation)	10.1	100 %
<i>C. vittelinum</i> (2 mg/ml)	7.4	73 %
<i>C. vittelinum</i> (5 mg/ml)	9.5	94 %
<i>O. lamiifolium</i> fraction A (2 mg/ml)	10.1	100 %
<i>O. lamiifolium</i> fraction A (5 mg/ml)	9.6	95 %
Acetaminophen (10 mM)	0.3	3 %
Acetaminophen + <i>C. vittelinum</i> (2 mg/ml)	3.4	34 %
Acetaminophen + <i>C. vittelinum</i> (5 mg/ml)	4.1	40 %
Acetaminophen + <i>O. lamiifolium</i> fraction A (2 mg/ml)	5.2	51 %
Acetaminophen + <i>O. lamiifolium</i> fraction A (5 mg/ml)	5.2	51 %

Table 6 : Effect of the aqueous decoctions on the duration of pentobarbital-induced sleeping time (CCl₄-intoxicated guinea pigs)

Plants	Sleeping time (ST) (min) (mean \pm SD; n = 8) ^(a)	% recovery ^(b)
Control pentobarbital (60 mg/kg)	144 \pm 3	-
Control CCl ₄ (Hepatotoxicant, 1.5 ml/kg)	300 \pm 5	-
<i>Crassocephalum vitellinum</i>	179 \pm 6 ***	77.9 %
<i>Guizotia scabra</i>	204 \pm 7 ***	61.4 %
<i>Microglossa pyrifolia</i>	297 \pm 6 ^{NS}	1.7 %
<i>Ocimum lamiifolium</i>	162 \pm 5 ***	88.2 %
<i>Vernonia lasiopus</i>	247 \pm 5 ***	34.1 %

(a) Treatments were compared by ANOVA with post-hoc pairwise comparisons (*t-test* with Bonferroni correction; *** : $p < 0.001$, NS : $p > 0.05$).

(b) % recovery = $\frac{\text{Hepatotoxicant ST} - \text{Antihepatotoxicant ST}}{\text{Hepatotoxicant ST} - \text{Pentobarbital ST}} \times 100$