

Hepatoprotective activity of extracts from *Landolphia kirkii*, *Uapaca kirkiana* and *Vitex madiensis* leaves' against Acetaminophen induced liver toxicity in rats

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Abstract:

Hepatoprotective activity of extracts from three plant species (*Landolphia kirkii*, *Uapaca kirkiana* and *Vitex madiensis*) was assessed against paracetamol-induced toxicity in male rats. These three plant species are among those used in Lubumbashi (DRC) to treat liver ailments.

Materials and Methods: Four solvents with different polarities (Hexane, chloroform, ethyl acetate and methanol) were used to obtain extracts from the leaves of these three plants. Male rats (n=4) weighing between 150 and 240 g, divided into 9 groups were treated (per os) with each of the plant extracts for 7 days, while paracetamol intoxication (1000 mg/kg) was carried out on the fifth day. Transaminase activity (AST and ALT) was measured to assess hepatoprotective activity.

Results: As the silymarin, all the extracts (methanolic and ethyl acetate extracts of *L. kirkii*, *V. madiensis* and *L. kirkii* and *U. kirkiana*) revealed anti-hepatotoxic activity against paracetamol ($p < 0.0001$). Among these extracts, ethyl acetate extract seems to be the best, with a hepatoprotection rate of 99.61%.

Conclusion: The results of this study demonstrate that *Landolphia kirkii*, *Uapaca kirkiana* and *Vitex madiensis* leaves' have a potent hepatoprotective action on acetaminophen induced liver damage in rats.

Key Word: Liver diseases, Hepatoprotective, Acetaminophen, Lubumbashi

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I. Introduction

The liver is one of the noble organs of the animal body. Due to its anatomical position and physiological role, it acts as a filter between pathogens of all kinds from the digestive mucosa and the systemic circulation. However, this original anatomical and immunological situation and the general detoxification functions it performs make this organ the preferred target of toxins resulting from interactions between the host and xenobiotics[1]. These pathogens include many drugs and industrial products to which the majority of the population is exposed, in this case industrialists and self-medicators[2].

Unfortunately, the treatments of choice for liver disease are controversial because conventional or synthetic drugs for the treatment of liver-related conditions are insufficient and sometimes cause serious side effects. As a

result, the incidence of liver diseases has not decreased or stopped; on the contrary, statistics show that they continue to increase[3].

In view of this situation, we set the general objective of contributing to the improvement of traditional medicine in the treatment of liver-related diseases. Specifically, we assess a hepatoprotective potent of three plants: *Landolphia kirkii*, *Uapaca kirkiana* and *Vitex madiensis*.

Landolphia Kirkii is native to tropical Africa and found mainly in Tanzania, Malawi, DRC, Uganda, Kenya and Zambia. It is generally found in tropical rainforests and savannah areas and one of fruit tree species found in Lubumbashi, in the south of DRC[4]. This plant, also known as the "Kirki rubber liana", is traditionally used for therapeutic purposes in certain regions of Africa[5].

The *Uapaca kirkiana* tree, known as Muzhanje in Shona, wild loquat in English and Masuku in Swahili, is an evergreen tree, some 5–6 m high, with rounded crown and rough grey bark. The fruit is a fleshy round belly, up to forty millimeters across, with a tough reddish brown skin surrounding yellow brown pulp in which several ridged seeds are embedded. The fruits are among the most popular in the south of DRC. The ripe fruits are sold at roadsides and at country markets. The pulp is honey – sweet with a slight flavour of oranges[6].

Vitex madiensis is a widely distributed plant in tropical Africa, growing on the periphery of open flora and savannah. This plant is called Rough Fingerleaf (English), locally (in DRC) it's called Mufutukinka, Filia (Kiyombe); Mfili (Kikongo), Mufutu (Luba); Kafutu-futu (Tshiluba)[7]; in West Africa: Dhumnia (Hausa); Galbihi (Peuhl); Kuti fingo (Malinké). *Vitex madiensis* is used to treat malaria and diabetes[8].

II. Material and Methods

- **Plant material**

The leaves of *U. kirkiana* and *V. madiensis* were collected from Mikembo Game Park areas, about 36 km from Lubumbashi while leaves of *L. kirkii*, were collected around Kipopo areas at about 25 km from Lubumbashi. All the leaves dried in hot air woven at 35°C for 12 days. The authentication of the plant was done at INERA (Institut National de Etude et de Recherche Agronomique).

- **Extraction of *Landolphia kirkii*, *Uapaca kirkiana* and *Vitex madiensis*' contents**

The leaves of all the three plants were air-dried and powdered, protected from light. The powder of each plant species was placed in a 2-liter percolator. A sufficient quantity of n-hexane was added until all the drug is immersed in the solvent. Keep macerated for 24 hours. Allow to run drop by drop, while continuously adding solvent until the solvent collected becomes discolored. Three other solvents were used under the same conditions: chloroform, ethyl acetate and methanol. Solutions obtained with ethyl acetate and methanol were concentrated under reduced pressure at 40°C to obtain ethyl acetate and methanolic extracts. Hexane and chloroform were used for delipidation.

- **Chemicals**

Acetaminophen and silymarin were supplied from MEPHARTECH. Aspartate aminotransferase(AST), alanine aminotransferase(ALT), were obtained from CYPRESS DIAGNOSTIC. Other chemicals used in this experiment were also of analytical grade.

- **Experimental animals**

Male Wistar albino rats, weighing about 180 – 240g were obtained from a veterinarian working for the Faculty of Veterinary Medicine at the University of Lubumbashi. Animals were kept in animal house at an ambient temperature of 25°C and 45 – 55% relative humidity, with 12 h each of dark and light cycles. Animals were fed pellet diet and water ad-libitum. All the experiment procedures were performed according to the purpose of control and supervision of experiments on animal (CPCSEA)[9].

- **Experimental procedure**

The rats were divided into nine groups each containing 4 rats[10].

Group-I: Control rats, which fed normal diet and water.

Group-II: Rats treated with acetaminophen (1000 mg /kg, per os, on the fifth day).

Group-III: Rats treated with Silymarin (200 mg/kg) orally once daily for 7 days + acetaminophen (1000 mg /kg, per os on the fifth day).

Group-IV: Rats treated with methanolic extract of *L. kirkii* (200 mg/kg) orally once daily for 7 days + acetaminophen (1000 mg /kg, per os on the fifth day).

Group-V: Rats treated with ethyl acetate extract of *L. kirkii* (200 mg/kg) orally once daily for 7 days + acetaminophen (1000 mg /kg, per os, on the fifth day).

Group-VI: Rats treated with methanolic extract of *U. kirkiana* (200 mg/kg) orally once daily for 7 days + acetaminophen (1000 mg /kg, per os on the fifth day).

Group-VII: Rats treated with ethyl acetate extract of *U. kirkiana* (200 mg/kg) orally once daily for 7 days + acetaminophen (1000 mg /kg, per os, on the fifth day).

Group-VIII: Rats treated with methanolic extract of *V. madiensis* (200 mg/kg) orally once daily for 7 days + acetaminophen (1000 mg /kg, per os on the fifth day).

Group-IX: Rats treated with ethyl acetate extract of *V. madiensis* (200 mg/kg) orally once daily for 7 days + acetaminophen (1000 mg /kg, per os, on the fifth day).

After the completion of the treatment, blood samples were collected from retro-orbital cavity of eye. The serum was used for the estimation of biochemical parameters.

- **Biochemical estimation**

The levels of aspartate aminotransferase(AST) and alanine aminotransferase(ALT) were estimated in the serum by using standard kits from CYPRESS DIAGNOSTIC.

- **Histopathological studies**

The livers were excised quickly and fixed in 10% formalin. Sections of about 4- 6 µm were stained with haematoxylin and eosin for histological evaluation. In brief 46 µm thick section of paraffin embedded rat liver were dewaxed with distilled water for 2 minutes. Then the section was stained with haematoxylin for 5 minutes at room temperature. After 15 minutes, the section was counterstained with eosin for 2 minutes, dehydrated with alcohol, washed with xylene and blocked by eosin. Hemotoxylin and eosin stained studies were observed under microscope.

- **Estimation of hepatoprotective rate (RHP)**

The hepatoprotection rate was calculated using the following formula:

$$HPR(\%) = \frac{(\Delta HT - \Delta x)}{\Delta HT} 100$$

For evaluation of enzyme activity:

ΔHT = difference in ASAT or ALAT activity between Control and Normal groups

Δ_x = difference in ASAT or ALAT activity between groups treated with plant species under study and the Normal group.

• Statistical analysis

Data for hepatoprotective activity were expressed as Mean $\pm$ SEM from four rats in each group. Hepatoprotective activity were analysed statistically using one-way analysis of variance (ANOVA), followed by Tukey Multiple Comparisons Test with the help of GRAPHPAD PRISM 8. P value of < 0.05 was considered as statistically significant.

III. Result and discussion

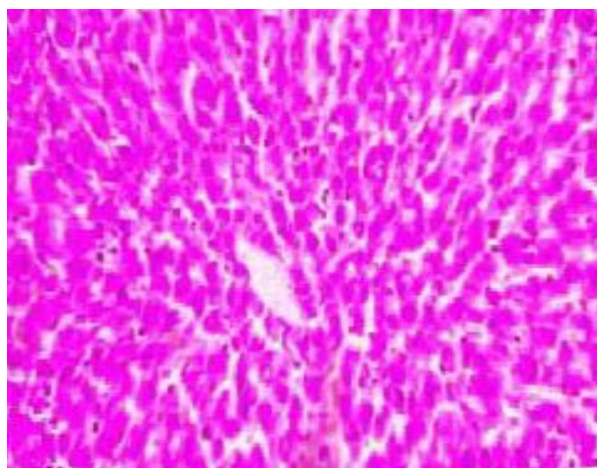
Measurement of biochemical parameters such as AST and ALT are used in the diagnosis and treatment of a variety of diseases involving the liver. After 7 days to study the hepatoprotective effect of *L. kirkii*, *U. kirkiana* and *V. madiensis* leaves, these biochemical parameters were measured. During experimental study we showed that in acetaminophen treated rats (Group-II) AST and ALT levels were sharply increased as compared to normal control group animals (Group I). Above results were indicated that severe liver damage occurred by acetaminophen [11]. AST and ALT levels were significantly decreased as compared to control group animals when treated, respectively with ethyl acetate and methanolic extracts of the three plants leaves' as well as the standard drug silymarin treated animals (Table 1).

Table 1: Effect of leaves' extracts of *Landolphia kirkii* Dryer, *Uapaca kirkiana* De Will and *Vitex madiensis* Oliv. in Acetaminophen (ACM) treated rats on serum transaminases

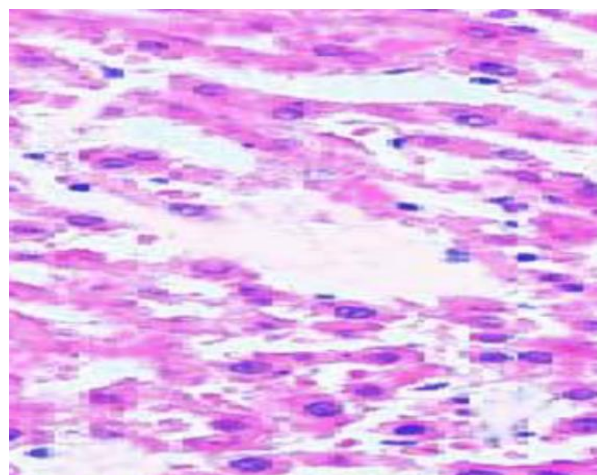
Group		AST		ALT	
		(UI/L)	RHP (%)	(UI/L)	RHP (%)
G-I	Normal control	60.00 $\pm$ 1.78***	-	53.09 $\pm$ 1.28 ***	-
G-II	ACM	227.51 $\pm$ 10.12	-	161.55 $\pm$ 8.10	-
G-III	Silymarin + ACM	61.26 $\pm$ 1.42 ***	99,25	60.22 $\pm$ 2.39***	93,43
G-IV	<i>L. kirkii</i> (MeOH) + ACM	86.75 $\pm$ 3.15***	84,03	61.17 $\pm$ 1.11***	92,55
G-V	<i>L. kirkii</i> (ACOET) + ACM	64.02 $\pm$ 7.00***	97,60	51.23 $\pm$ 2.6***	101,71
G-VI	<i>U. kirkiana</i> (MeOH) + ACM	119.07 $\pm$ 9.92***	64,74	123.75 $\pm$ 10.43***	53,29
G-VII	<i>U. kirkiana</i> (ACOET) + ACM	127.04 $\pm$ 12.3***	59,98	128.62 $\pm$ 5.90 ***	58,02
G-VIII	<i>V. madiensis</i> (MeOH) + ACM	134.08 $\pm$ 9.29***	55,72	138.19 $\pm$ 6.76 ***	55,65
G-IX	<i>V. madiensis</i> (ACOET) + ACM	79.11 $\pm$ 6.19***	88,59	79.51 $\pm$ 4.02 ***	75,64

Legend: MeOH= methanolic extract; ACOET= Ethyl acetate extract; *** = $p < 0.0001$ when all groups are compared to ACM group.

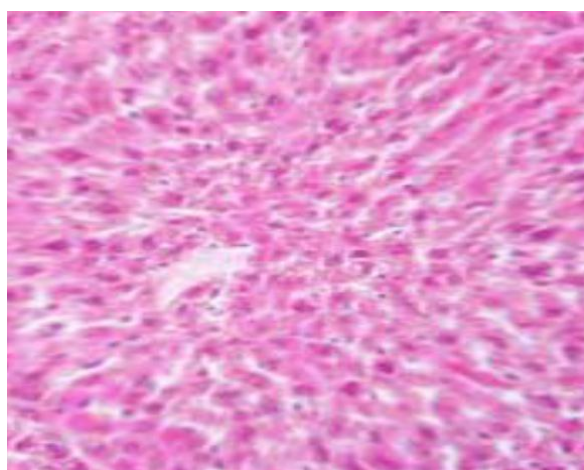
Histopathological studies showed that the normal control rats had normal hepatocytes and central veins. Necrosis of normal hepatocytes, inflammatory cell and congestions were observed in ACM treated animals. The liver sections of the rats treated with silymarin, MS extract and its fractions followed by acetaminophen showed a sign of healing which was indicated the absence of necrosis. All results are compared with the silymarin + ACM treated animals (Group-III).



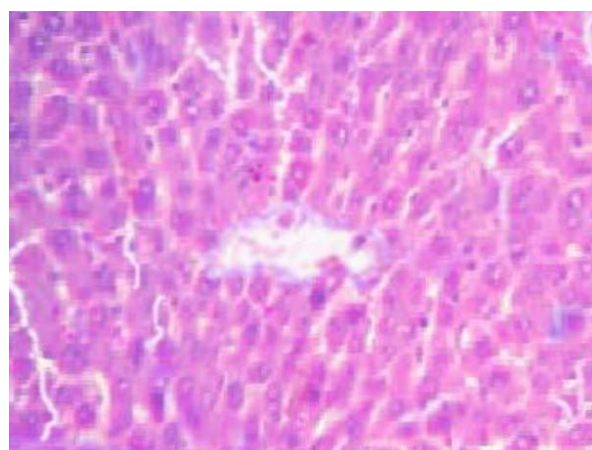
Group I : Normal



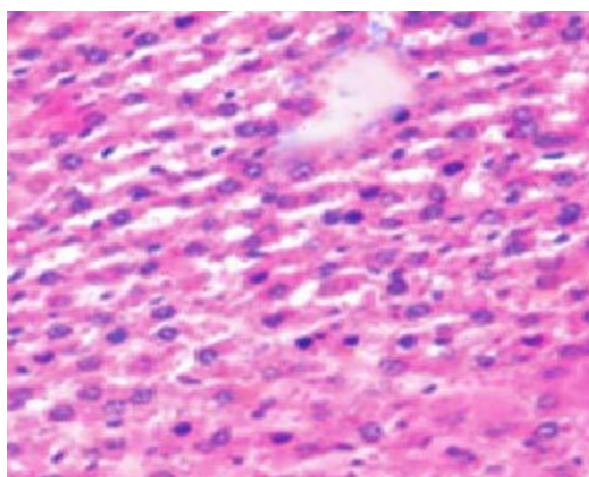
Group II : Acetaminophen (ACM)



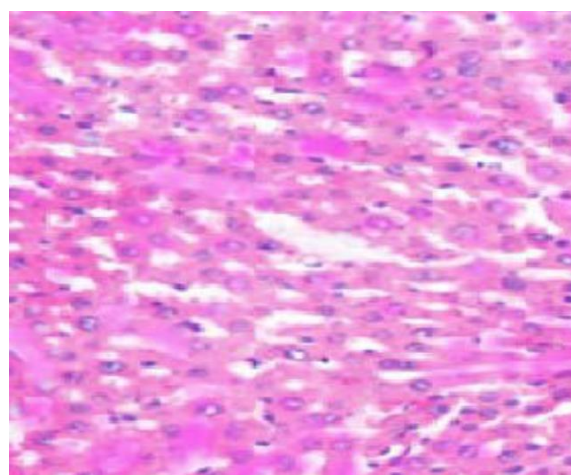
Group III : Silymarin + ACM



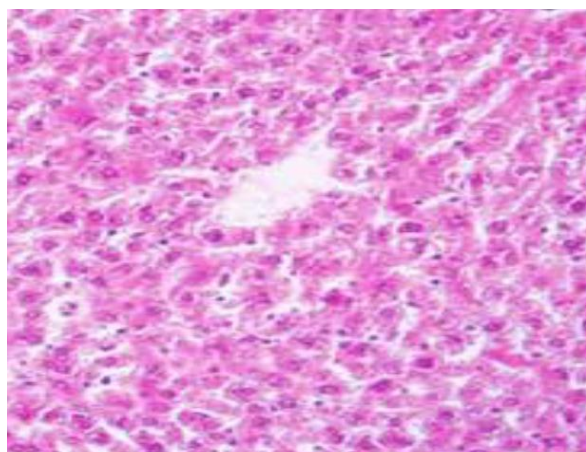
Group IV : *L. kirkii* (MeOH) +



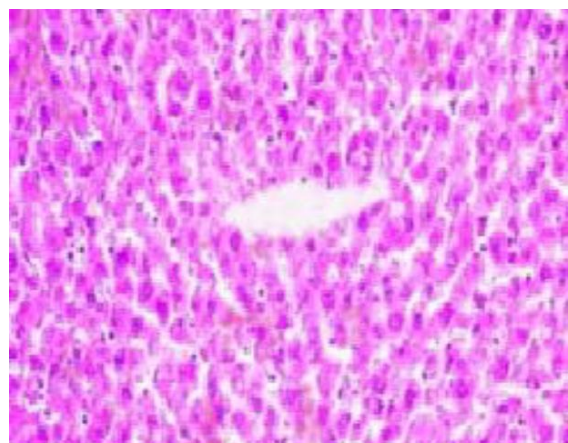
Group-V : *L. kirkii* (ACOEt) + ACM



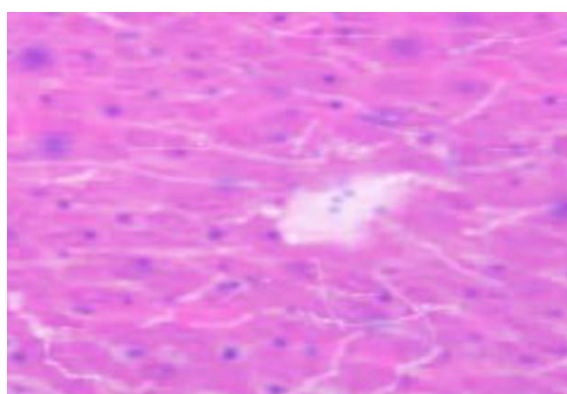
Group-VI : *U. kirkiana* (MeOH) + ACM



Group-VII : *U. kirkiana* (ACOEt) + ACM



Group-VIII : *V. madiensis* (ACOEt) + ACM



Group-IX : *V. madiensis* (MeOH) + ACM

This study evaluates the hepatoprotective role of methanolic and ethyl acetate extracts of *Landolphia kirkii*, *Uapaca kirkiana* and *Vitex madiensis* leaves against acetaminophen - induced hepatotoxicity in albino rats. Paracetamol (acetaminophen) is widely consumed as an antipyretic drug that is safe in therapeutic doses but can cause fatal hepatic damage in human and animal at higher toxic doses[12]. Administration of paracetamol at a dose of 1-3 gm/kg/day/p.o. results in hepatic damage. The toxic metabolite N-acetyl-pbenzoquinimine is an oxidative product of paracetamol formed by the action of cytochrome P-450 and it reacts with reduced glutathione (GSH) to yield non-toxic 3-GSyl-paracetamol. Depletion of GSH causes the remaining quinone to undergo covalent bonding with cellular macromolecules (sulphydryl groups of protein) and leads to cell death[13].

The liver damage causes leaking of cellular enzymes into the plasma due to the disturbance of hepatocytes transport functions[14]. A group of enzymes to be found in cytosol is discharge into the blood when liver cell plasma is damaged. So causing increased enzyme levels in the blood serum. An obvious sign of hepatic injury is the leaking of cellular enzymes such as ALT, AST and ALP into plasma due to the disturbance caused in the transport functions of hepatocytes. ALT is more specific to the liver, and it is a better parameter for analyzing hepatic injury[15].

High levels of AST indicate the cellular leakage as well as loss of functional ability of cell membrane in liver[16]. After 7 days' treatment with Acetaminophen AST and ALT levels were estimated. There was a sharp rise in above biochemical parameters in Acetaminophen treated experimental group. The elevated levels of AST and ALT in this study may be interpreted as a result of the hepatocytes damage or alterations in the membrane permeability indicating the severity of hepatocellular damage induced by ACM[17]. However, the AST and ALT levels were significantly decreased in all the groups where animals were treated with *L. kirkii*, *U. kirkiana*

and *V. madiensis*' extract five days before Acetaminophen administration (Table 1). The above results were well comparable with Acetaminophen + silymarin treated groups.

The rate of hepatoprotection was determined in order to compare the hepatoprotective powers of the different products administered to the rats. The results showed that, with ALT as a marker, the ethyl acetate extract of *L. kirkii* completely annihilated the hepatotoxicity of ACM, with a RHP of 101%, better than silymarin (93.43%), the known reference product. It was followed by the methanolic extract of the same plant (92.55%), and the ethyl acetate extract of *V. madiensis* (75.04% of RHP). The methanolic extract of *U. kirkiana* provided the lowest RHP (53.29%).

Even with AST as a marker, the ethyl acetate extract of *L. kirkii* was better (97.60%), but the lowest activity was that of the methanolic extract of *V. madiensis*, with a RHP of 55.72%.

The results of the present work indicate that *Landolphia kirkii*, *Uapaca kirkiana* and *Vitex madiensis* can protect the liver against Acetaminophen induced hepatotoxicity.

IV. Conclusion

The results of this study demonstrate that *Landolphia kirkii*, *Uapaca kirkiana* and *Vitex madiensis* leaves' have possess a potent hepatoprotective action on acetaminophen induced Liver damage in rats. However, further detailed studies are required to isolate the active compound(s) and to establish its clinical application.

What this study adds

Among the numerous publications on *in vivo* hepatoprotective activity, none were reported in the DRC. Here's the first one.

In this investigation, a comparison of hepatoprotective power was carried out.

Competing interests

The authors declare no competing interests.

Author's contributions

Faustin Mwamba Maseho is the principal investigator who prepared this manuscript. Salvius Bakari Amuri has made the necessary contacts for the acquisition of reagents and other consumables. Marsi Mbayo Kitambala and Valentin Bashige Chiribagula contributed to the adaptation of the article for submission. Sylvestre Ngoy Mulopwe contributed to the revision of the manuscript and the adaptation of the article for submission. Serge Mbuyi Kalonji has devoted himself particularly to the study of *Landolphia kirkii* Nathalie. Nathalie Mwenze Musenge, Joel Kabadi Kasongo, Biayi Benaja Martin and Vianney Ntabaza Ndage assisted in the biological analysis as well as proofreading the manuscript. Cédric Mutombo Shakalenga was responsible for the statistical processing of the results. Richard Kalunga Muya contributed to the bibliographic review of plants with hepatoprotective activity. Kahumba Byanga is the initiator of this research project on plants with hepatoprotective activity. Jean-Baptiste Lumbu Simbi is the supervisor of our entire research team on medicinal plants used in traditional medicine in Lubumbashi and its surroundings.

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