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CHEMICAL SCREENING AND EFFECTS OF AQUEOUS EXTRACT OF FRESH LEAVES OF MORINDA LUCIDA BENTH ON MARKERS OF OXIDATIVE STRESS AND LIVER FUNCTION IN LPS-TREATED BALB/C MICE

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ABSTRACT

Background: The use of *M.L BENTH* extracts in many countries of the sub-region illustrates its beneficial and multiple effects in the management of many pathologies. However, in the Republic of Congo despite its use in rural communities, no study has attempted to elucidate its chemical composition and its effects on liver function. This study examined the chemical composition and effects of the aqueous extract of fresh *Morinda lucida BENTH* leaves on markers of oxidative stress and liver function in Balb/c mice treated with LPS. ***Methods:*** Fresh *Morinda lucida BENTH* leaves were collected, carefully washed, dried at room temperature and powdered before maceration. The main phytochemical families of the aqueous extract of these leaves were sought. Balb/c mice (n = 5) weighing 16 to 28 g respectively, were treated per os orally with different concentrations of this extract (200 mg/kg and 400 mg/kg) “group D and E” then intraperitoneally with LPS while distilled water “group A” was used as a negative control, distilled water associated with LPS “group B” and Donepezil 5 mg/kg “group C” as positive controls. ***Results:*** Phytochemical analysis revealed that the aqueous extract of fresh leaves of *Morinda lucida BENTH* contains alkaloids, tannins, flavonoids and glycosides "sugars". The values of the oxidative stress and liver function parameters studied showed that the aqueous extract of *M.L BENTH* leaves does not influence the functioning of these markers of oxidative stress: SOD and NO, as well as those of liver function: TG and GPT, for the study groups (B, C, D, E); CT for the study groups (C, D, E); and GOT for the study groups (B, C, D): ($p>0.05$) compared to the control group (A). In addition, those of CT for the study groups (B: $*p<0.03$; C: $*p<0.04$)) and GOT for the group (E: $*p<0.03$) show a significant difference compared to the control group (A). ***Conclusions:*** The present study showed that the aqueous extract of *M.L BENTH* leaves has bioactive chemical compounds; and that at the doses studied, the aqueous extract of *M.L BENTH* leaves does not influence the desired markers of oxidative stress. Similarly, the aqueous extract of *M.L BENTH* leaves at a dose of 200mg/kg does not influence the desired markers of liver function, unlike the dose of 400mg/kg of the aqueous extract of *M.L BENTH* leaves which influences GOT; which would require further and appropriate research since some bioactive compounds could be used for pharmacological purposes.

KEYWORDS: *Morinda lucida; leaf extract; phytochemical; oxidative stress; liver function.*

INTRODUCTION

Plants are naturally known to contain bioactive chemical compounds , some of which can be toxic.[1] Many xenobiotics are likely to cause damage to the proper functioning of organs.[2] The damage is often localized at the hepatic level due to the central role that the liver plays in the metabolism of xenobiotics, as well as its anatomo-physiological and portal position in the bloodstream.[3]

A wide range of phytochemicals possessing natural molecules with anti-infective properties, acting directly on the pathogen or the owner by stimulating the innate and acquired defense mechanisms of the host.[4] Thus, drug discoveries focused on molecules with multiple

actions can contribute to the management of pathologies and/or the prevention of the emergence of resistance. In the search for multi-action molecules, *Morinda lucida BENTH* has been identified for its many therapeutic effects.[5]

Morinda lucida BENTH., a plant species of the Rubiaceae family; known as the sulfur tree, is a traditional plant commonly used in West Africa. In Nigeria, extracts of these leaves are used in the treatment of malaria as well as in the control of blood sugar.[6 ; 7 ; 8] The combination of these leaves and root barks and/or roots has strong hypoglycemic, antidiabetic, anticancer, hepatoprotective, antimicrobial, antidiarrheal properties.[9; 10; 11]

In the Republic of Congo, several rural communities use infusions of different parts of *Morinda lucida BENTH* for these various therapeutic properties, although this is not documented. In the "northern" basin, *Morinda lucida BENTH* commonly called ossika, is frequently used by rural communities in the treatment of headaches, fever and malaria. However, no scientific data at the national level have been reported so far on its chemical composition and its impact on liver function. In this study, we investigated the main chemical families present in the aqueous extract of fresh leaves of *M.L BENTH* as well as its influence on markers of oxidative stress and liver function in LPS-treated Balb/c mice.

MATERIAL AND METHODS

Plant material and preparation of the aqueous extract

Fresh leaves of *Morinda lucida BENTH.* They were collected in the city of Makoua (Cuvette department) on May 3, 2018 at 4 p.m. They were identified and registered under number N°: 8.014 on June 6, 2018 at the National Herbarium of Congo, IRSEN in Brazzaville. These leaves were reduced to powder using the electronic grinder; 50g of powder obtained were macerated in 500ml of distilled water under magnetic stirring for 48 hours. The maceration obtained was filtered using carded cotton and then concentrated to a quarter (1/4) of its initial volume at 65°C in an oven. The pure extract obtained was transferred to a sterile plastic container; hermetically sealed and stored in the refrigerator at 4°C for psychopharmacological analyses.[12]

Animal material and treatment

We used Balb/c mice, male and female, weighing 16 to 28 g respectively (age between 7 – 10 weeks). They came from the animal house of the Faculty of Health Sciences. These animals were kept in their natural habitat and subjected to a 12/12h light/dark cycle, with free access to water and food. These animals were divided into five (5) groups of five (5) Balb/c mice per group and treated for 7 days orally then with LPS for groups (B, C, D, E) for 3 days by IP (intraperitoneal injection) as follows: group A, treated with distilled water 10ml/kg; group B, treated with distilled water then with LPS; group C, treated with Donepezil 5 mg/kg then with LPS; group D, treated with aqueous extract of *M.L BENTH* leaves 200mg/kg then with LPS; and group E, treated with aqueous extract of *M.L BENTH* leaves 400mg/kg then with LPS.[13]

Blood samples

The animals were anesthetized with chloroform "Ether cooper bottle 125 ml" one hour after the last dose of treatment. Blood was collected at the orbital level using capillary micropipettes and at the jugular level. The samples were centrifuged at 3000 rpm for 15 minutes and the supernatants/sera were collected in 2ml tubes, stored in the refrigerator at -4 °C for biochemical analyses.

Phytochemical study[14, 15]

Chemical screening was used to identify the chemical families (alkaloids, flavonoids, tannins and reducing sugars) in the leaves of *M.L BENTH.* To identify the presence of alkaloids, 1 ml of 1 N hydrochloric acid and a few drops of Dragendorff's reagents were added to 5 ml of aqueous extract of *Morinda lucida BENTH* leaves, and a red precipitate was observed indicating the presence of alkaloids. In a test tube containing 5 ml of aqueous extract of *Morinda lucida BENTH* leaves, 1 ml of the aqueous solution of iron II chloride was added, and a greenish or blue-blackish coloration was observed indicating the presence of tannins. 5 ml of the hydrochloric acid (HCL) solution, 1 ml of isoamyl alcohol and a few magnesium shavings were added in 5 ml of aqueous extract of *Morinda lucida BENTH* leaves; A variation of red coloration was observed, indicating the presence of flavonoids "flavonols and flavanols". The revelation of reducing sugars was recorded by the appearance of a brick red precipitate by adding 1 ml of Fehling's solution in 5 ml of aqueous extract of *Morinda lucida BENTH* leaves.

Serum parameters

Serum biochemical parameters were measured using commercial Cypress Diagnostic kits (Langdorpsesteenweg 160. 3201 langdorp. Belgium www.diagnostics.be) on the CYAN® spectrophotometer. The colorimetric kinetic method was used to determine serum concentration of total cholesterol, triglycerides (TG). GOT and GPT concentrations were determined by the kinetic method according to IFCC. Liquid.

ELISA

The level of SOD and NO secretion in serum was measured by the Sandwich-ELISA method, using ELISA kits (Sunlongbiotech; www. Sunlongbiotech.com) according to the supplier's recommendations. Sera from treated animals were diluted with standard dilution samples present in the ELISA kits. OD was read at 450 nm using an Autobio PHOmo microplate reader. The results obtained were multiplied by the dilution factor: x 5.

STATISTICAL ANALYSIS

The results are expressed as mean ± SEM. The significance threshold was P < 0.05; and it was calculated by the student t test using IBM SPSS Statisticts 23 software.

RESULTS

Phytochemical analyses of the aqueous extract of *M.L BENTH* leaves reveal the presence of the chemical families sought, namely alkaloids, tannins, flavonoids and sugars. This identification made it possible to note in the aqueous extract of *M.L BENTH* leaves, a very abundant quantity of flavonoids, an abundant quantity of alkaloids, tannins and sugars/glycosides. Table I presents these results encountered.

The values of CT concentrations for study groups (D, E), GOT for groups (B, C, D), TG, GPT, SOD and NO studied for groups (B, C, D, E) did not show statistically significant differences ($p>0.05$) compared with the control group (A). In addition, the results of CT for study groups (B: $*p<0.03$; C: $*p<0.04$) and GOT for group (E: $*p<0.03$) show a significant difference compared with the control group (A). These results are illustrated in Table II.

Table I: Chemical families identified in the aqueous extract of M.L BENTH leaves.

Chemical family	*Alkaloids*	*Tannins*	*Flavonoids*	*Sugars/glycosides*
Indication	+++	+++	++++	+ + +

Indication : +++ = abundant quantity, ++++ = very abundant quantity.

Table II: Effect of the aqueous extract of M.L BENTH leaves on biochemical parameters in Balb/c mice (n = 5).

Parameters	*Water Dist.10ml/Kg*	*LPS + Water Dist.10ml/Kg*	*LPS+Donépezil 5mg/kg*	*LPS + M.L BENTH 200mg/Kg*	*LPS + M.L BENTH 400mg/Kg*
CT (mmol/l)	*1.40±0.03*	*1.10±0.07**	*1.65±0.11**	*1.29±0.19*	*1.45.34±0.14*
TG (mmol/l)	*0.74±0.17*	*0.92±0.21*	*1.00±0.19*	*1.17±0.23*	*1.26±0.33*
GPT (UI/l)	*63.35±9.37*	*43.50±8.28*	*48.40±6.24*	*58.10±15.53*	*61.71±9.26*
GOT (UI/l)	*81.39±9.86*	*100.26±24.06*	*65.65±24.11*	*94.16±17.78*	*123.10±6.79**
SOD (pg/ml)	*101.44±7.93*	*104.80±0.46*	*103.76±3.30*	*106.80±4.84*	*108.80±0.85*
NO (pg/ml)	*27.96±1.99*	*27.06±2.44*	*29.12±1.80*	*29.64±3.24*	*24.60±0.83*

DISCUSSION

The investigation of chemical screening is the first step to know the active molecules present in a plant material. Knowledge of the different chemical families present in a plant species allows it to be classified according to its bioactive power. In our study, we looked for the chemical families namely, alkaloids, tannins, flavonoids and sugars/glycosides. In the same vein, the work of OO Adeleye, OJ Ayeni and MA Ajamu in 2018 on "the traditional and medicinal use of Morinda lucida" highlighted the presence of a wide range of chemical families namely alkaloids, tannins, steroidal flavonoids, terpenoids, saponosides, phenols, hydrogen cyanide and glycosides; in the leaves, bark and roots of the said plant. Their synthesis work highlighted the very high presence of phenols; the high presence of alkaloids, tannins, flavonoids; the lower presence of steroids and glycosides; the presence in the form of traces of terpenoids, saponins and hydrogen cyanide. Ebiloma Godwin Unekwuojo, Omale James and Aminu Rhoda Olubunmi in 2011, in their research on the "Suppressive, curative and prophylactic potentials of Morinda lucida (Benth) against the erythrocyte stage of infectious mice sensitive to chloroquine Plasmodium berghei NK-65" found as a result a predominance of alkaloids and flavonoids. By comparing with the results of the leaves encountered, our study on the aqueous extract of *M.L BENTH*, allowed us to identify a very abundant quantity of flavonoids, an abundant quantity of alkaloids, tannins and sugars/glycosides. These results confirm the existence of these large chemical families in the leaves of *M.L BENTH*.[16]

The values of the parameters of oxidative stress and liver function studied showed that the aqueous extract of *M.L BENTH* leaves does not influence the functioning of these markers of oxidative stress: SOD and NO, as well as those of liver function. : TG and GPT, for study groups (B, C, D, E); CT for study groups (D, E); and GOT for study groups (B, C, D), compared to the control group (A). In addition, the parameters of liver function: CT for study groups C and B; GOT for study group E, showed a significant difference compared to the control group (A). Oduola Taofeeq and al. evaluated in 2010 a large number of parameters of liver function of albino wistar rats exposed to *Morinda lucida* leaf extracts. This work allowed them to conclude that the ingestion of Morinda lucida leaf extracts has no toxic effect on liver function parameters. The results of our study are similar to those of the work of Oduola Taofeeq et al.; in 2010 on "the evaluation of hepatotoxicity and nephrotoxicity of albino wistar rats exposed to Morinda lucida leaf extracts".[17]

CONCLUSION

This study showed that the aqueous extract of *M.L BENTH* leaves has bioactive chemical compounds; and that at the doses studied, the aqueous extract of *M.L BENTH* leaves does not influence the markers of oxidative stress sought. Similarly, the aqueous extract of *M.L BENTH* leaves at a dose of 200 mg/kg does not

influence the sought markers of liver function, unlike the dose of 400 mg/kg of the aqueous extract of *M.L BENTH* leaves which influences GOT among the sought markers of liver function.

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Conflict of interest
No conflict of interest.

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