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Phytochemical study, evaluation of the hyperglycaemic activity and acute toxicity of mn, an anti-diabetic phytomedicine sold in Korhogo

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Abstract

Diabetes is a growing public health issue in Côte d'Ivoire, linked to urbanization, sedentary lifestyles, and dietary changes. Many patients turn to traditional medicine. This study investigated MN, an antidiabetic herbal product from Korhogo composed of *Anogeissus leiocarpus*, *Aloe vera*, *Chrysanthellum indicum*, and *Monodora tenuifolia*. Phytochemical screening (in vitro, TLC) of its ethanolic extract revealed alkaloids and polyphenols. NMR spectroscopy (¹H, ¹³C, COSY, HMBC, NOESY) identified (+)-magnoflorine, a bioactive aporphine alkaloid. Acute toxicity testing (OECD 423) indicated MN is non-toxic (LD₅₀ > 2000 mg/kg bw). In glucose tolerance tests, oral MN (140 mg/kg bw) significantly lowered blood glucose levels, comparable to metformin (3 g/kg bw). These findings support MN's antidiabetic potential and traditional use in Côte d'Ivoire.

Keywords: Traditional medicine, (+) magnoflorin, antihyperglycemic, acute toxicity

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from either insulin deficiency or peripheral resistance to its action [1]. There are two main types of diabetes: type 1, which is usually diagnosed in children and adolescents, and type 2, which is much more common in adults and often associated with lifestyle factors. Its prevalence is constantly increasing, making it a real public health problem in both developed and developing countries [2].

According to recent data published in *The Lancet*, more than 800 million adults worldwide currently have diabetes, a fourfold increase since 1990 [3]. In Côte d'Ivoire, prevalence rose from 5.7% in 1997 to 6.2% in 2017 [4], and WHO projections estimate that in Africa, the number of cases could reach 47.1 million adults aged 20 to 79 by 2047 [5]. This rapid increase is putting considerable pressure on health systems already struggling with other communicable diseases.

In addition to its impact on health, diabetes places a heavy economic burden on society. The costs associated with its management include not only direct expenses for medication and medical care, but also indirect costs such as lost productivity and absenteeism. In low- and middle-income countries, these financial burdens contribute to the impoverishment and deterioration of patients' quality of life. Despite the proven effectiveness of modern treatments, a growing proportion of the population is turning to traditional medicine, which is perceived as more accessible [6].

However, the chemical composition, therapeutic efficacy, and potential toxicity of many traditional remedies remain poorly documented. In Korhogo, Côte d'Ivoire, an antidiabetic herbal medicine known as MN is commonly sold. It combines four medicinal plants: *Anogeissus leiocarpus* (Connaraceae), *Aloe vera* (Asphodelaceae), *Chrysanthellum indicum* (Asteraceae), and *Monodora tenuifolia* (Annonaceae). Previous studies have reported the antihyperglycemic properties of *Aloe vera* and *Anogeissus leiocarpus*, particularly through improved insulin sensitivity and reduced oxidative stress [7, 8]. Other studies have also confirmed the antidiabetic activity of each of these four species [9, 10, 11, 12].

This study, the first of its kind to our knowledge on this herbal medicine, aims to characterize its chemical constituents, evaluate its antihyperglycemic activity, and determine its acute toxicity in order to better document its pharmacological profile.

Materials and Methods

Material

Plant material

The MN medicine was purchased in Korhogo (Ivory Coast) from a traditional medicine practitioner in January 2024. According to this practitioner, it is an equal mixture of four plants, namely *Anogeissus leiocarpus* (root bark), *Aloe vera*, *Chrysanthellum indicum* (leaves), and *Monodora tenuifolia* (seeds), which comes in liquid form. The dosage is one glass of tea twice a day.

Animal equipment

Swiss albino female mice weighing between 19 and 26 grams were used to assess acute toxicity.

Albino male and female rats weighing between 160 and 200 grams were used to assess antidiabetic potential.

These animals came from the animal facility of the Faculty of Pharmaceutical and Biological Sciences at Félix Houphouët-Boigny University (Ivory Coast). They were fed pellets supplied by FACI® (Fabrication d'Aliments Composés Ivoiriens) and had free access to water. The animals were acclimatized in spacious, hygienic plastic cages lined with wood shavings and kept at an ambient temperature of 26 ± 1 °C, with a relative humidity of $50 \pm 5\%$ and a 12-hour light-dark cycle.

Chemicals and solvents

The chemicals and solvents are composed of: ethanol, distilled water, Burchard's reagent, Dragendorff's reagent, silica gel 60 F254 chromatography plate, ethyl acetate, methanol, iron(III) chloride, acetic acid, chloroform, cyclohexane, dichloromethane, physiological solution (0.9% NaCl), glucose (Pharmivoire®, Ivory Coast), ether (Cooper®), metformin (Glucophage®).

Methods

Obtaining powder from MN

The traditional medicine MN, initially in liquid form, was heated to 50°C in order to remove the solvent. The powder obtained was then stored in boxes for various analyses.

Phytochemical screening

Preparation of the extract for phytochemical screening

The MN powder was dissolved in 60% ethanol. After filtration, the MN ethanol solution obtained was used to search for certain specific metabolites.

Search for specific metabolites

The search for specific metabolites (alkaloids and polyphenols) was carried out using qualitative tube analysis methods and thin-layer chromatography (TLC).

Alkaloids

Testing for alkaloids in tubes

The methods used are those described by Békro *et al.* (2007) [13]. Alkaloids were detected using Burchard's reagent (iodo-iodide reagent) and Dragendorff's reagent (potassium iodo-bismuthate reagent). Thus:

- Two (2) drops of Dragendorff's reagent are added to 2 ml of the MN ethanol solution, and the appearance of a precipitate or orange color indicates the presence of alkaloids.
- Two (2) drops of Burchard's reagent are added to 2 ml of the MN ethanol solution, and the appearance of a reddish-brown precipitate indicates a positive reaction.

Search for alkaloids by CCM

The stationary phase is a silica gel 60 F254 chromatographic plate with a rigid aluminum support. The developing agent (mobile phase) or migration solvent used is a mixture of ethyl acetate and methanol in a ratio of 90:10 (v/v). A few drops of the MN ethanol solution are deposited using a capillary tube on a point on the baseline, drawn 1 cm from the bottom of the chromatographic plate. After drying the extract deposit, the plate was placed in the saturated tank containing the migration solvent mixture. After developing the phytochemicals, the plate is dried and then sprayed with Dragendorff's reagent. All orange or red colors observed in the visible spectrum correspond to alkaloids [14].

Polyphenols

Test tube polyphenol research

To detect polyphenols, 2 mL of the MN ethanol solution is added to a few drops of a 2% (m/v) aqueous solution of iron (III) chloride (FeCl₃). The appearance of a blue-black or green-black color indicates the presence of polyphenols [14, 15].

Search for polyphenols by CCM

After pipetting the MN ethanol solution onto the chromatographic plate as before, it is placed in a tank containing the acetic acid/chloroform migration system in the respective proportions (1:9) (V/V). After development, the plate is placed under a UV lamp at 254 nm and 365 nm. The appearance of a blue-black or green-black color indicates the presence of polyphenols [16].

Purification and isolation of KAB.MC.A77

Obtaining compound KAB.MC.A77

12 g of MN powder was purified on a silica gel column by introducing cyclohexane, chloroform, ethyl acetate, and methanol, respectively. This initial roughing allowed seven fractions (KAB.MC.1 to KAB.MC.7) to be grouped according to their chromatographic profile. Fraction KAB.MC.7 was purified on Sephadex®LH-20 gel using a binary mixture of MeOH/ CH₂Cl₂ (1/1) as the elution solvent. This purification led to the isolation of compound KAB.MC.A77 (7.9 mg).

Nuclear Magnetic Resonance (NMR)

This spectroscopy technique is applied to particles or groups of atomic particles that have a non-zero nuclear spin. All Nuclear Magnetic Resonance (NMR) analyses were performed using the following experiments:

One-dimensional experiments: ¹H, ¹³C *J*-Mod (*J* Modulation Spin Echo) and two-dimensional experiments: COSY (Correlated Spectroscopy), HSQC (Heteronuclear Single Quantum Correlation), HMBC (Heteronuclear Multiple Bond Correlation) and NOESY (Nuclear Overhauser Enhancement and Exchange Spectroscopy). The spectra were recorded on a Bruker-Avance 400 instrument (¹H 400 MHz, ¹³C 100 MHz) with a 5 mm inverse multinuclear (*Z* gradient) BBI probe. Chemical shifts are expressed in ppm using deuterated solvent as a reference (CD₃OD). Coupling constants (*J*) are expressed in Hertz (Hz). To describe signal morphology, the following abbreviations are used to express multiplicity: “*s*” for singlet, “*d*” for doublet, “*t*” for triplet, “*q*” for quadruplet, and “*m*” for multiplet. All assignments presented have been confirmed by two-dimensional experiments (HSQC, HMBC, COSY, NOESY).

Acute toxicity

Evaluation of the acute toxicity of MN powder on mice

This study was conducted to determine the toxicity level of the MN drug product and to ascertain whether the different

doses of the extracts contained any contaminants or foreign elements. During the experiments, we used OECD Protocol 423 [17]. Each step required the use of three (03) mice. The doses chosen for the different MN extracts prepared were: 5, 50, 300, and 2000 mg/kg body weight (Fig. 1). The toxicity level selected was that at which mortality could be expected to occur among some of the treated animals. The choice of animal sex (female) is required by the guideline, due to the high sensitivity of this type of mouse. The animals in each test batch were each force-fed at specific observation times (every 30 min). When the treated batch showed no dead animals and no other signs of toxicity (aggression, mobility, alertness, stool condition, vomiting, mortality, etc.), the next batch was then treated in turn. The animals were observed on the first day and regularly every day for 14 days.

Method of administration

After fasting the mice for 24 hours, they were force-fed using a rigid tube. The technique is based on a simple principle. It involved holding the mouse firmly so that it did not move

during administration. Once the tube was inserted into the animal's throat, the extract was also injected gently. The mice were treated in five groups of three. Each group underwent the following treatment:

- **Group I (control):** animals received distilled water,
- **Group II (treated):** animals received 5 mg/kg body weight of extract,
- **Group III (treated):** animals received 50 mg/kg body weight of extract,
- **Group IV (treated):** animals received 300 mg/kg body weight of extract,
- **Group V (treated):** animals received 2000 mg/kg body weight of extract.

After treatment, the mice were observed individually every hour on the first day and regularly every day for two weeks (14 days). The animals' behavior and clinical symptoms were noted throughout the experiment, and their body weights were measured every three days until the end of the experiment.

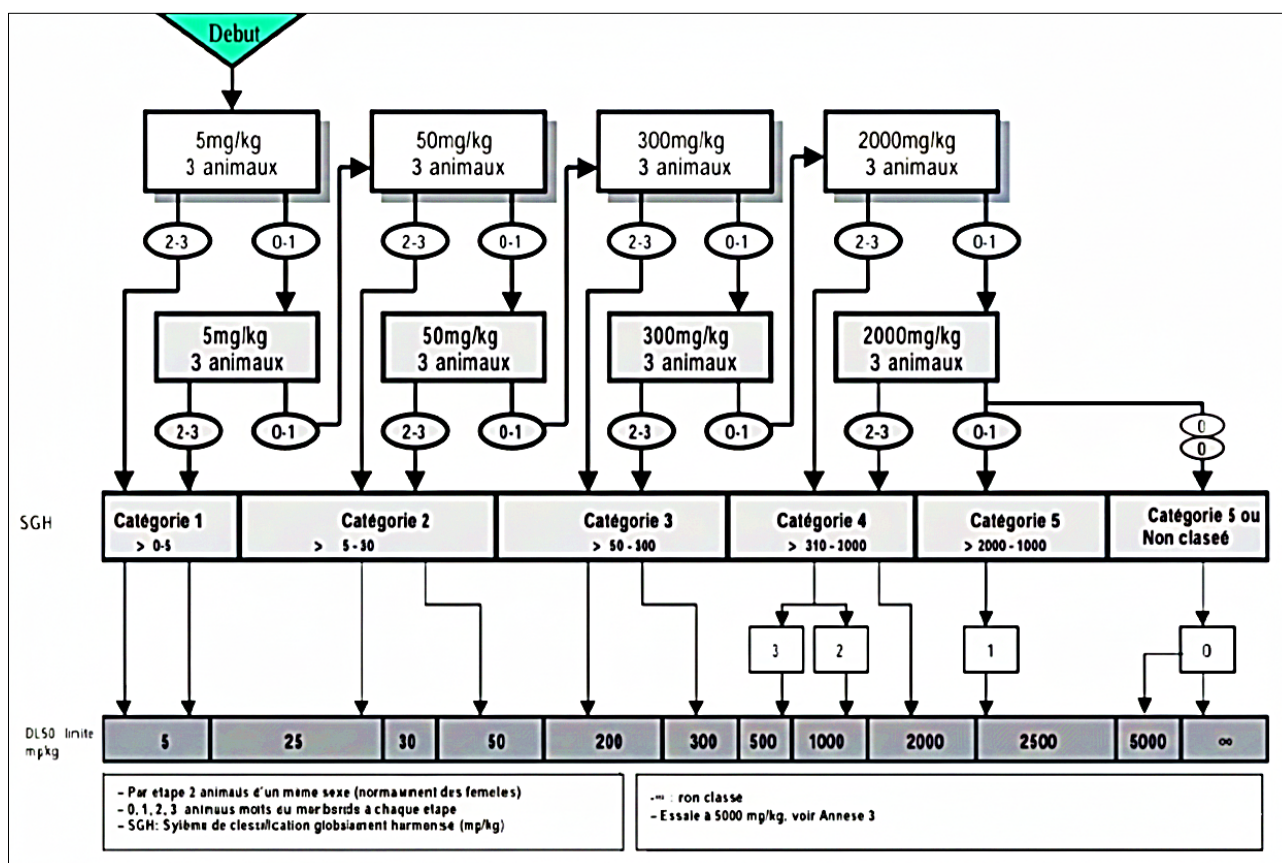


Fig 1: Test scheme showing the inoculation of extract doses administered to mice [17]

Anti-hyperglycemic activity

The test was performed according to the method described by Kambouche *et al.* (2011) [18].

Principle

The test consisted of measuring baseline blood glucose levels, administering an oral glucose overload at a dose of 3 g/kg bw, then treating the animals with the various study solutions (0.9% NaCl, 100 mg/kg metformin, Extracts: 30, 70, and 140 mg/kg) and monitoring blood glucose levels for 90 minutes (T30, T60, T90). A substance is said to be anti-hyperglycemic if it is capable of reducing hyperglycemia.

Procedure

The rats, fasted for 16 hours, were divided into six homogeneous groups according to weight using the following procedure:

- **Group 1:** hyperglycemic rats receiving physiological solution (10 ml/kg body weight per os) = control NaCl 0.9%;
- **Group 2:** hyperglycemic rats receiving metformin (10 mg/kg body weight per os) = metformin reference;
- **Group 3:** hyperglycemic rats receiving macerated extract 1 (35 mg/kg body weight per os) = test batch 1;

- **Group 4:** hyperglycemic rats receiving macerated extract 1 2 (70 mg/kg bw orally) = Test batch 2;
- **Group 5:** hyperglycemic rats receiving macerated extract 1 (140 mg/kg bw orally) = Test batch 3;

In each group, at T0, the baseline blood glucose level of each rat was measured, then all rats in the same group were given an overload of a 3g/kg glucose solution to induce hyperglycemia. At T30, T60, and T90, blood glucose levels were measured again using a glucometer.

The variation in baseline blood glucose was determined using the following formula^[19]:

$$\text{Variation (\%)} = \frac{G_t - G_0}{G_0} \times 100$$

- **G_t:** Blood glucose level at time t after administration of substances to rats
- **G₀:** Baseline blood glucose levels in fasting rats at time T₀

The antihyperglycemic activity expressed as a percentage reduction at time t was determined using the following

formula^[18]:

$$\text{Reduction (\%)} = \frac{(\text{VMGT} - \text{VMGE})}{(\text{VMGT})} \times 100$$

- **VMG_t:** Average blood glucose level of the control
- **VMG_E:** Average blood glucose value during the trial

Data analysis

The results, expressed as mean ± standard deviation in each batch, will be compared using the Kruskal-Wallis test followed, in the event of a significant difference, by Bonferroni's post hoc test, at a risk of $\alpha = 0.05$.

Ethical constraints

The experimental procedures were conducted in accordance with good experimental practices and in compliance with OECD European guidelines.

Results

Phytochemical screening

Phytochemical tests on MN revealed that the traditional medicine contains phenolic and alkaloid compounds. The various results obtained are recorded in Table 1.

Table 1: chemical screening results for MN

Specialized metabolites	MN
Alkaloids	+
Polyphenols	+

The results are expressed according to the type of reaction: Positive: +; Absence: -

Structural elucidation of KAB.MC.A.77, (+)-magnoflorine

Compound KAB.MC.A.77 is a yellow amorphous solid. The ESI (positive) mass spectrum (Fig. 2) showed a molecular peak at m/z 342 $[M]^+$, corresponding to the molecular formula $C_{20}H_{24}NO_4$. The compound obtained reacted positively with Dragendorff's reagent, suggesting that it is a nitrogen-containing derivative. Analysis of the two-dimensional homonuclear 1H - 1H (COSY and NOESY) (Fig. 3) and heteronuclear 1H - ^{13}C (HSQC and HMBC) HMBC (Fig. 4) correlation spectra suggests an aporphine-type structure.

The 1H NMR spectrum (Fig. 5) shows signals from two methoxyl groups. The protons of the different methoxyl groups resonate at δ_H 3.82 $[OCH_3]$ and 3.85 ppm $[OCH_3]$. These protons are attributable to protons in positions 2 (C-2) and 10 (C-10), respectively. Analysis of the proton spectrum also shows the presence of three aromatic protons at δ_H 6.91, 6.93, and 7.00 ppm (H-3, H-8, H-9), two methoxyl groups at δ_H 3.82 and 3.85 ppm, and two N-methyl groups resonating at δ_H 2.85 $[N-(CH_3)]$ and 3.36 ppm $[N-(CH_3)]$. These various observations are corroborated by the study of the ^{13}C NMR spectrum (Fig. 6). Indeed, it indicates the presence of three aromatic carbons at δ_C 110.9; 119.8 and 111.9 ppm, two methoxyl groups at δ_C 56.4 and 56.5 ppm, and two N-methyl groups at δ_C 43.3 and 53.4 ppm. Analysis of the spectrum shows 2J and 3J correlations between the aromatic proton at δ_H 6.51 ppm and the carbons at δ_C 141.8 (C-1);

120.1 (C-3a); 23.6 (C-4) and 120.4 ppm (C-1b). This correlation allows this proton to be placed in position 3 of the aporphine cycle A. Other correlations are also visible between the protons at δ_H 2.47 ppm (H-7 α); 3.25 (H-5 α) with the δ_C carbons at 119.8 ppm (C-8) and 43.3 ppm $[N-CH_3$ (1)], respectively. These correlations allow the various methylene groups to be placed on ring B. Another correlation can also be observed between the proton at δ_H 6.52 ppm and the carbons at δ_C 30.2; 111.9; 149.2, and 121.4 ppm on the one hand, and between the proton at δ_H 6.71 ppm and the carbons resonating at δ_C 119.8 and 142.2 ppm on the other. These correlations allow these different protons to be placed in positions 8 and 9 of the C cycle (Table 2). The different positions of the methylene groups and protons 8 and 9 are confirmed by analysis of the COSY spectrum. Indeed, correlations between the protons of the methylene groups (H-4 α and H-5 α) on the one hand, and between the aromatic protons (H-8 and H-9) visible on the COSY spectrum on the other. The different protons are carried by adjacent vertices. The absolute configuration S of carbon 6a is deduced from the positive sign of the optical rotation (+ 24.3). The consistency of all these spectral data allows us to propose the structure of (+)-magnoflorine for compound KAB.MC.A77^[20] (Fig. 7). This compound has already been isolated from an Annonaceae, *Xylopiya parviflora*^[21] and from *Monodora crispata*^[22].

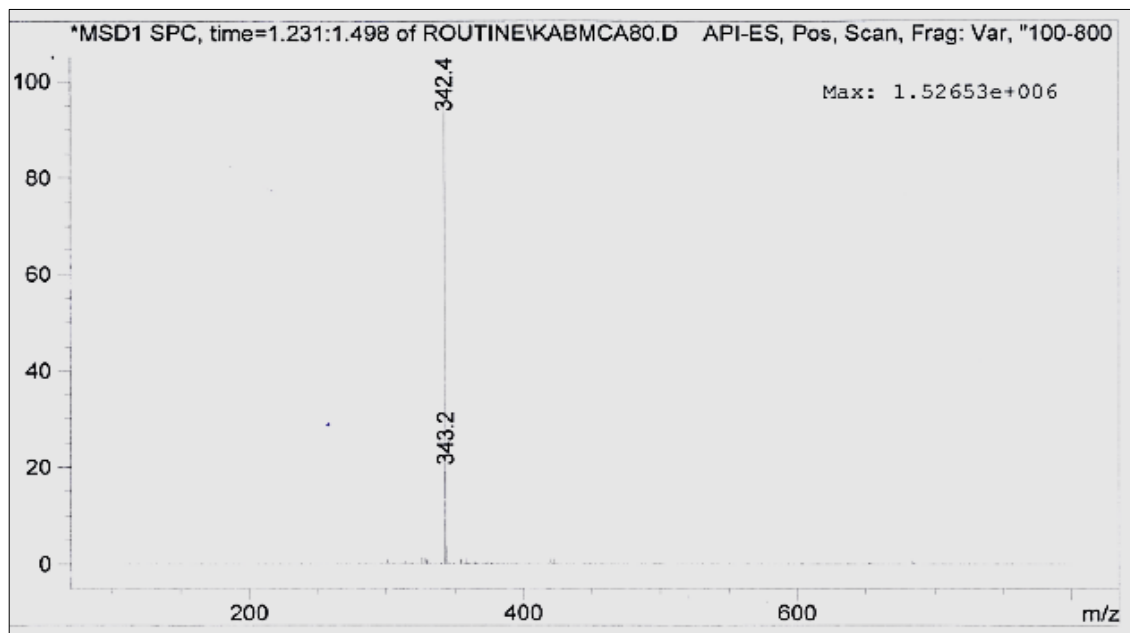


Fig 2: ESI mass spectrum (positive) of KAB.MC.A.77 ((+)-magnoflorine)

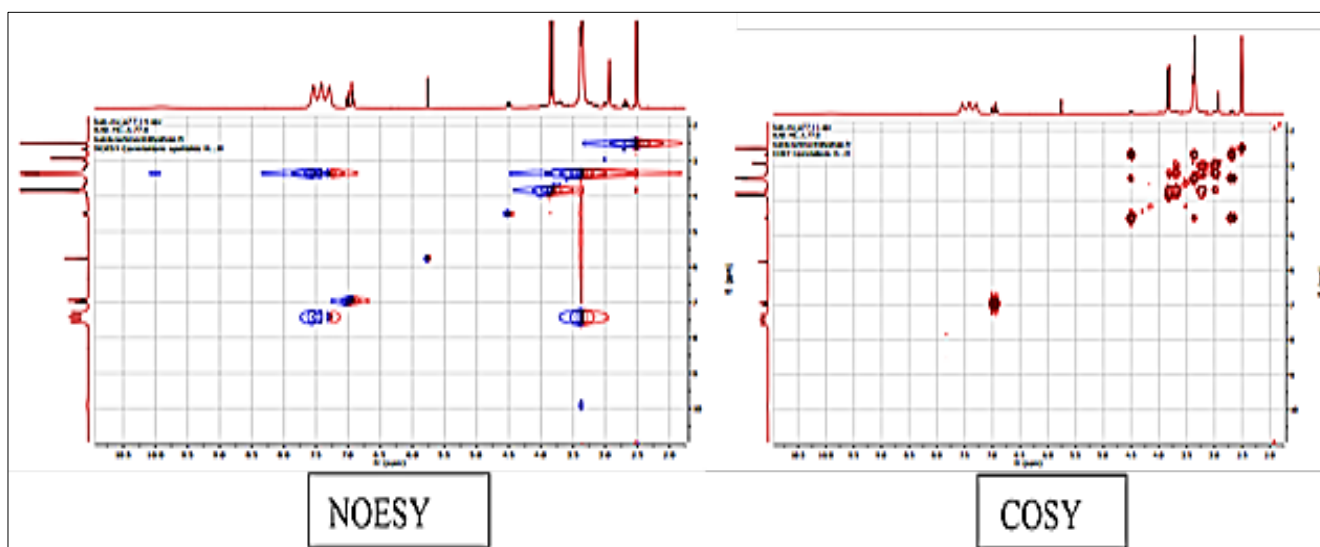


Fig 3: COSY and NOESY spectra of KAB.MC.A.77 ((+)-magnoflorine)

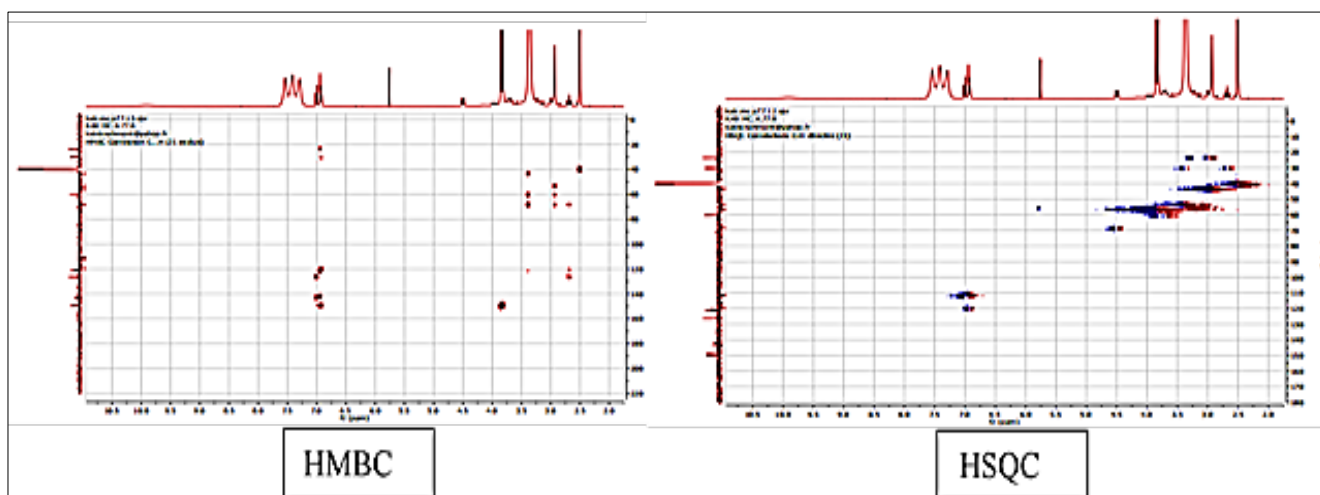


Fig 4: HMBC and HSQC spectrum of KAB.MC.A.77 ((+)-magnoflorine)

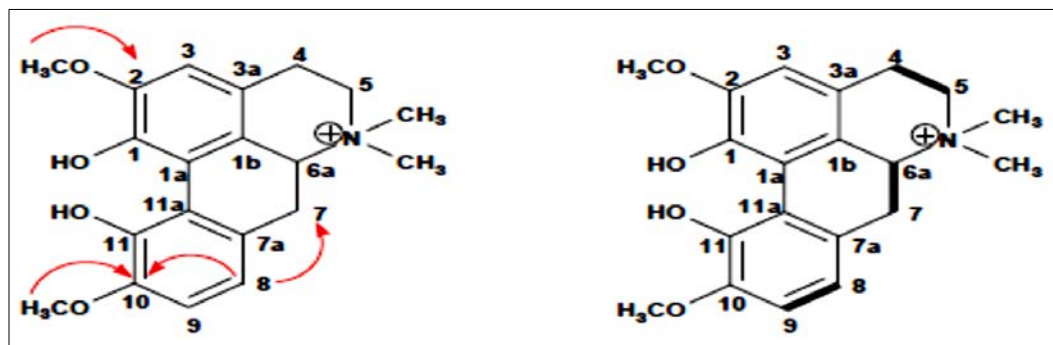


Fig 6: HMBC (red) and COSY (black) correlations of (+)-magnoflorine

Acute toxicity assessment

Observations conducted over a 24-hour period, then daily for two weeks, on the control groups of mice and those treated with the various extracts revealed no toxicity. No signs of toxicity were observed in the control mice that received only distilled water. They remained in good physical condition, with healthy skin, fur, eyes, and mucous membranes. No abnormal behavior (diarrhea, salivation, lethargy, aggression,

drowsiness) was noted, their heart rate remained stable, and their mobility was normal. No deaths were recorded. In all cases, the treated mice showed a state of health comparable to that of the controls, with no abnormal behavior or mortality recorded throughout the study (Table 3). Thus, the drug MN is non-toxic at 2000 mg/kg. Consequently, its lethal dose 50% (LD₅₀) is greater than 2000 mg/kg bw.

Table 3: Observations over 24 hours and 2 weeks of control and treated mouse groups

Observations	Sample Lot		Experimental Lots	
	6 hours	12 hours	6 hours	12 hours
Skin and fur	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Mucous membranes	Normal	Normal	Normal	Normal
Diarrhea	Absence	Absence	Absence	Absence
Salivation	Absence	Absence	Absence	Absence
Lethargy	Absence	Absence	Absence	Absence
Heart rate	Normal	Normal	Normal	Normal
Aggression	Absence	Absence	Absence	Absence
Drowsiness	No	No	No	No
Feeding	Yes	Yes	Yes	Yes
Mobility	Yes	Yes	Yes	Yes
Mortality	No	No	No	No

Antihyperglycemic activity: Glucose tolerance test

Figure 8 shows the evolution of hyperglycemia after

administration of the MN extract.

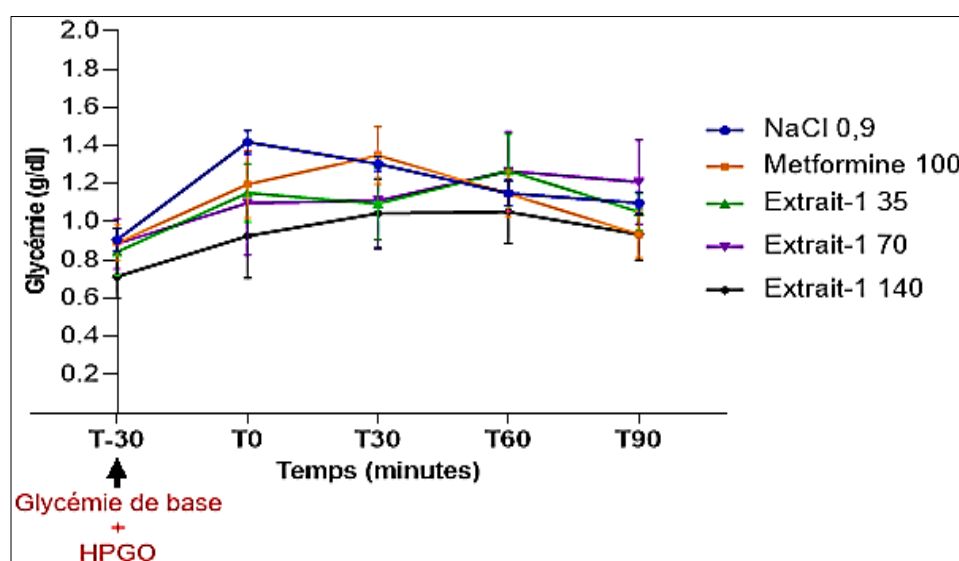


Fig 8: Antihyperglycemic activity of MN

The administration of an oral glucose overload at a dose of 3 g/kg bw caused a significant increase in blood glucose levels after 30 minutes. MN was administered at several

concentrations. The extract at 140 mg per kg body weight showed the same result as the reference molecule Metformin (Fig. 8).

Discussion

Phytochemical screening revealed that MN ethanolic extract contains alkaloids and polyphenols, two metabolic classes associated with antihyperglycemic and antioxidant properties [8, 23].

Structural elucidation by NMR (^1H , ^{13}C) and two-dimensional analyses (COSY, HMBC, NOESY) identified (+)-magnoflorine, an aporphine alkaloid already described for its hypoglycemic and antioxidant effects [23, 24].

The evaluation of antihyperglycemic activity showed that at 140 mg/kg bw, MN significantly reduced post-oral glucose load blood glucose levels, with efficacy comparable to that of metformin, the first-line drug for type 2 diabetes [25]. These results show that MN has antidiabetic activity, as described by traditional medicine practitioners. The antidiabetic activity claimed by traditional medicine practitioners could be due to the presence of (+)-magnoflorine. This compound is a quaternary aporphine-type alkaloid. This molecule has already been isolated from *Sinomenium acutum*, *Coptis chinensis*, and *Xylopiia parviflora*. It has several pharmacological activities, including antidiabetic, anti-inflammatory, immunomodulatory, hypotensive, antioxidant, and antifungal activities [23]. Given its beneficial pharmacological properties, magnoflorine should be a potential drug candidate for the treatment of diabetes, depression, or Alzheimer's disease. In China, certain herbal medicines such as *Cuscuta chinensis* and *Berberis kansuensis* contain magnoflorine. These plants are widely used to treat diabetes. A multitude of studies have shown that magnoflorine has antidiabetic activity. Researchers have found that magnoflorine can stimulate insulin secretion in the RINm5F cell line, and oral administration of magnoflorine (40 mg/kg) significantly reduced fasting blood glucose levels [26].

Since magnoflorine is an antioxidant, antidiabetic, and anticholinergic agent, it could also be used to treat glaucoma. In addition, molecular docking was performed to understand the interactions between magnoflorine and the target enzymes BChE (D: 6T9P), hCA II (A:3HS4), AChE (B:4EY7), and α -glycosidase (C:5NN8). The results suggest that magnoflorine could be an important compound in the transition from natural sources to industrial applications, particularly new drugs [27].

In addition, the four plants involved have already been shown individually to have antidiabetic activity. Previous studies report the hypoglycemic effects of *Aloe vera* and *Anogeissus leiocarpus* in animal models [7, 8].

In a situation of prolonged hyperglycemia (diabetic state), the total extract and supernatant fraction of *Anogeissus leiocarpus* roots showed strong antidiabetic potential by reducing hyperglycemia, glucose intolerance, and hyperlipidemia in diabetic Sprague Dawley rats. In addition, the total extract increased β -cell function and showed significant antioxidant properties. These activities are related to the bioactive compounds present, which can react alone or synergistically [9]. Among all the medicinal plants used to treat diseases, the genus *Aloe* is one of the most common. They can be used to treat various conditions, including inflammation, infections, cancer, and diabetes mellitus. There are specific reports on the hypoglycemic and antidiabetic potential of different species of *Aloe* [10]. Data from the literature confirm the many uses of *Chrysanthemum indicum* in traditional medicine. Scientific studies have confirmed its antioxidant, anti-inflammatory, antidiabetic, and antipyretic properties, confirming its long-standing use [11]. Oxidative stress has been implicated in the

pathogenesis of many diseases, including diabetes mellitus, due to the excessive production of free radicals. Therefore, *Monodora tenuifolia* seeds are good therapeutic agents that can alleviate complications related to oxidative stress in diabetes mellitus [12].

From a toxicological standpoint, MN did not cause any mortality or clinical signs of toxicity at a dose of 2000 mg/kg bw, which classifies it as a low-toxicity substance according to OECD 423 (2001) [17]. The herbal medicine is non-toxic and could therefore be consumed safely by the populations of Korhogo.

Conclusion

This study highlights that MN, a traditional antidiabetic herbal medicine, contains alkaloids such as (+)-magnoflorine and polyphenols, whose biological activity confers a significant antihyperglycemic effect. MN has low acute toxicity ($\text{LD}_{50} > 2000$ mg/kg bw) and efficacy comparable to metformin in the glucose tolerance test.

These results support the promotion of MN as a promising herbal medicine for diabetes mellitus. Future investigations should include chemical standardization, pharmacokinetic studies, chronic toxicity studies, and clinical trials to confirm its safety and efficacy in humans.

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