

Full Length Research Paper

Toxicological and phytochemical screening study of *Mitragyna Inermis* (willd.) O ktze (Rubiaceae), anti diabetic plant

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Mitragyna inermis leaves are used in Ivorian traditional medicine to treat diabetes. To situate tolerance limits, we determined acute toxicity threshold by the Overall Harmonized System of classification (SGH) using predetermined dose method and then we highlighted principal chemical groups responsible for pharmacological activity. The results allowed us to conclude that any dose of *M. inermis* leaves aqueous extract (300, 2000 and 5000 mg/kg) is lethal for the inoculated animals. LD₅₀ is thus higher than 5000 mg/kg. These results suggest that the aqueous leaf extract of *M. inermis* could be used with some degree of safety by oral route. Phytochemical analysis revealed the presence of sterol, triterpene, polyphenol, flavonoïd, catechic tannin, saponoside and alkaloid, which conferred several pharmacological activities on the leaves of the plant.

Key words: *Mitragyna inermis*, acute toxicity, lethal dose, GHS class, phytochemistry.

INTRODUCTION

African populations are confronted with chronic diseases emergence whose treatment and follow-up constitute for them more economic problem. Diabetes belongs to these new diseases for African traditional medicine. Unknown in the old days, these diseases developed until becoming a real public health problem.

According to W.H.O., the number of diabetes patients in the world was 177 million in 2000; this number could reach 300 million by the year 2025 (Anonymous, 2007a). In Africa, about 14 million of people suffer from diabetes, and this number should double from now to 2025 (Anonymous, 2007b). One of the more alarming points is the fast increase in the number of people from 30 to 50 years developing diabetes.

In Ivory Coast, 3 to 7% of diabetics were diagnosed in hospitals (Lokrou, 1980; Kadja, 1998; Djédjé, 2002; N'guessan, 2008). But this situation is below of reality because many patients do not attend hospitals. Insulin dependant diabetes (Diabetes of type1) represent for 20 to 25% of the whole cases of diabetes observed against 75% for Non Insulin dependant diabetes (Diabetes of type 2).

Because of its chronicity, gravity, complications and means which must be implemented for its treatment, diabetes is an expensive disease, not only for the patient and his family, but also for medical authorities.

According to some studies in India, an Indian family with low income whose adult member suffer of diabetes devotes 25% of his resources to take their patient into care. In United States of America, this number is 10% for families having a diabetic child (Anonymous, 2007a).

The number of death allotted to diabetes was estimated before at a little more than 800,000 per year, but we

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known that this number was for a long time largely underestimated. Really, it is probably located in the region of 4 millions deaths per year, either 9% of total mortality (Oga et al., 2006; Sy and Cissé, 2007). The search for a therapy which can help to overcome diabetes definitively remains actually a major concern for modern medicine.

In view of the expansion of this disease whose minimum fare is raised, WHO in its resolution AFR/RC50/R3 of August 31, 2000, encouraged African countries of which Ivory Coast, to develop regional strategies on traditional medicine in order to begin research on medicinal plants and to promote their optimal uses in health service systems.

The present study is a preliminary work which contributes to a better knowledge on properties of medicinal plants used in traditional medicine in Ivory Coast for diabetes treatment, notably *Mitragyna inermis*.

Several plant leaves formulations are proposed to patients (decoction, infusion, maceration, mixture) and the administration doses vary from one tradipraticians to the other. Thus, it is pressing to develop a scientific approach in order to situate the plant tolerances limits. Several kinds of compounds including polyphenols, sterols, triterpene saponins, anthraquinones, lignans, flavonoids, alkaloids, tannins are biological or pharmacological activity and its interesting to known the leaf plant chemical composition for optimization its medicine utilization (Kerharo et al., 1974; Gharras et al., 1999; Jun and Ryan, 2005; N'guessan, 2008).

MATERIAL AND METHODS

Plant material

Vegetable material is constituted by leaves of *M. inermis* (Willd.) O. Ktze (Rubiaceae). This plant has as synonym *Uncaria inermis* Willd., *Mitragyna africana* (Willd.) Korth., *Nauclea africana* Willd.). It is a shrub or small tree from 8 to 10 m height, with many stood up stems on the basis, smooth and greyish barks with clearly brown section, going darker in the light. Elliptic leaves from 7cm out of 4cm with short petiole, reds when young with 6 to 7 pairs of secondary nervures (Aké-Assi, 1984; ACCT, 1985; Lebrun et al., 1997).

The plant leaves have been collected in Korhogo area (North of Ivory Coast). Leaves are dried in an oven and crushed finely under laboratory temperature conditions. The powder obtained will constitute our sample to be analyzed. One part of leaves powder obtained is freeze-dried for toxicity test and the other part was used for phytochemical screening study.

Animals

For this study white mice were provided by vivarium of higher teacher training school and then kept in animalery of the Training and Research Unity of Pharmaceutical and Biological Sciences at University of Abidjan-Cocody. They are 4 to 8 weeks old. The mice were fed with FACI (Fabrication d'Aliments de Côte d'Ivoire) pellets, groundnuts and dried fish. Their drink was tap water. A total of 21 mice were used in this study.

Toxicological study

Aqueous extract preparation

The leaves powder is freeze-dried and a solution of 15 mg/ml is prepared with distilled water and used for animals force-feeding. A volume of 2 ml/100g of body weight is used. Mice were maintained into fasting for about 3 to 4 h before extract administration without water deprivation, and then they were normally fed 2 h later. Then, animals were weighed, marked and dispatched in order to have homogeneous batches compared to the weight.

Acute toxicity study

This work is based on determination of the leaves toxicity class of the plant used traditionally in diabetes treatment by the overall harmonized system of classification which uses predetermined dose method (Anonymous, 1984; Chenu et al., 1987).

Acute oral toxicity (Ecobichon, 1997; Kumar et al., 2008) study was performed as per OECD-423 guidelines (acute toxic class method). Mice of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg body weight by intragastric tube (OCDE, 1998) and observed for 14 days according to OCDE (2001) recommendations. If mortality was observed in 2 - 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50 and 300 mg/kg body weight (OCDE, 2001). Otherwise, if amount 300 mg/kg body weight did not prove to be toxic, higher dose (2,000 and 5,000 mg/kg) were used to determine the toxicity of plant. Ultimately, five groups appear:

- Group 1dose 5 mg/kg (0,25 mg/ml)
- Group 2dose 50 mg/kg (2,5 mg/ml)
- Group 3dose 300 mg/kg (15 mg/ml)
- Group 4dose 2,000 mg/kg (100 mg/ml)
- Group 5dose 5,000 mg/kg (250 mg/ml)

All experiments were repeated 3 times.

Phytochemical screening

It consists in identifying for a plant, chemical compounds groups showing pharmacological interest. The different extracts obtained with the powder were used to identify and characterize some chemical groups.

Preparation of the extract

The collected leaves were air dried at room temperature until it attained a constant weight. The dried leaves were then powdered using pestle and mortar as follows;

* 20 g of the powder was extracted three times in 60 ml of chloroform for 15 min. The solution is filtered on gauze. This process is repeated twice and extract was filtered using a cheese cloth. The filtrate was over a sand bath to achieve the concentration of 25 ml solution (Solution I or chloroform solution)

* 60 ml of methanol are added on the previously dried marc. We shake up during 15 min. This operation was repeated twice and extract was filtered and concentrated as previously (Nemlin et al., 1995; Konaté, 1997; Gnalei, 2005), Solution II or methanol solution.

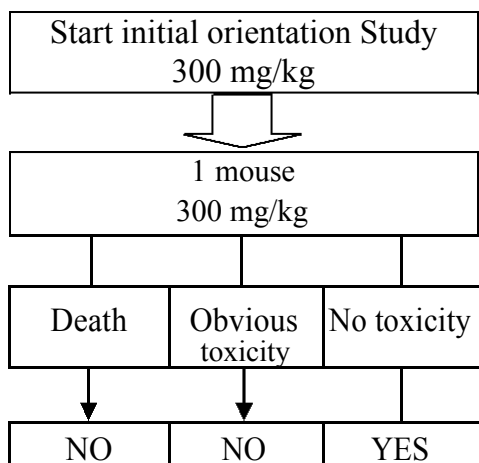


Figure 1. Dose administration in orientation study by predetermined dose method result.

* 5 g of the powder was extracted in 50 ml of boiling water for 15 min. The extract was filtered using a cheese cloth (Nemlin et al., 1995). The filtrate represents the infused solution or aqueous extract N°1 (solution III). * 100 g of powder was extracted in 1,0 l of distilled water for 25 min using a hot plate. The decoction extract was filtered as above and represents the aqueous extract N°2 (solution IV).

Phytochemical analysis

The different solutions have been characterized to determine the various major chemical groups present in the leaves of *M. inermis*. All analyses were repeated three times. Thus, the following methods were used:

- Bouchardat and Dragendorff for alkaloids (Nemlin et al., 1995; Rafael and Elena, 2005).
- Reaction of Liebermann for steroids (Brock et al., 2006; N'Guessan, 2008).
- Reaction with ferric chloride for polyphenols (Rafael et al., 2005; N'Guessan, 2008).
- Reaction called "with cyanidine" for flavonoïds (Nemlin et al., 1995; Brock and Herzfeld, 2006; N'Guessan, 2008).
- Reaction of Stiasny for tannins (Nemlin et al., 1995; Rafael et al., 2005; Brock and Herzfeld, 2006).
- Reaction of Borntraeger for quinoid compounds (Gbeassor al., 1989; Nemlin and Brunel, 1995; N'Guessan, 2008).

RESULTS

Acute toxicity studies

This study showed no mortality up to different doses administered to mice (Figures 1 and 2). So, the extracts safe for long term administration.

Mice are individually observed at least once during first 30 min and regularly during the first 24 h after *M. inermis* leaves freeze-dried aqueous extract administration by force-feeding. We did not notice any particular clinical sign and any death during the 14 days of observation.

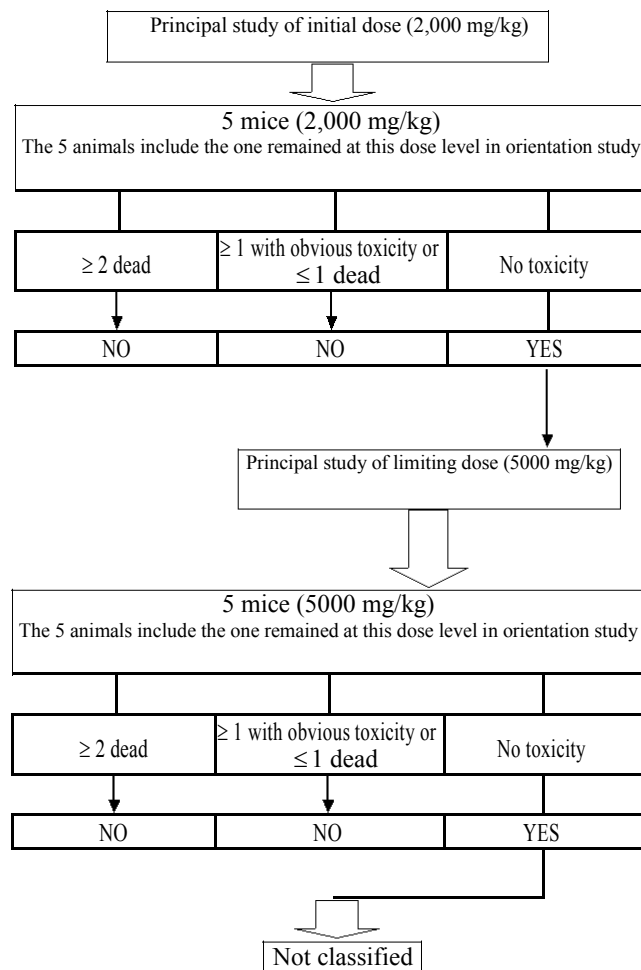


Figure 2. Principal study by a predetermined dose of 2,000 mg/kg administration then a limit dose of 5000 mg/kg.

Phytochemical screening

Principal chemical groups identified are consigned in the Table 1. The result showed that polyphenols, flavonoïds, catechic tannins are present in methanolic, infused and decocted leaves extracts but not in chloroformic extract. Saponosides are only present in decoction, sterols, triterpenes and alkaloids are present in all leaves extracts, contrary to gallic tannins and quinoid which are absents in every extracts.

DISCUSSION

The predetermined dose method does not aim at precise value of LD₅₀ calculation, but determines product SGH category (Anonymous, 1984; OCDE, 1998; 2001) involving acute toxicity. We used a predetermined initial dose of 2,000 mg/kg for principal test after an orientation study that did not shown any mortality with doses of 300 mg/kg body weight and 2,000 mg/kg. A limit dose of 5,000 mg/kg body weight was appreciated by this same pre-

Table 1. Phytochemical analysis.

Identified chemicals groups	Leaves powder extract			
	Chloroform Solution 1	Methanol Solution 2	Infusion Solution 3	Decoction Solution 4
Sterols and triterpenes	+	+	+	+
Polyphenols	-	+	+	+
Flavonoids	-	+	+	+
Catechic Tannins	-	+	+	+
Gallic Tannins	-	-	-	-
Quinoid substances	-	-	-	-
Saponosides	-	-	-	+
Alkaloids	+	+	+	+

(+) Present ; (-) Absent.

determined dose. In test case (predetermined dose method), SGH classification indicates as result to consider either a classification in group 5 {2,000 mg/kg (100 mg/ml) < category 5 5,000 mg/kg (250 mg/ml)} or a non SGH classification for substance involving acute toxicity. According to directives indications on predetermined dose method for the test substance, could be classified in SGH group of danger 5, substances defined by 2,000 mg/kg body weight (100 mg/ml) < group 5 5,000 mg/kg (250 mg/ml), in the following cases:

- If, on the basis of mortality incidence, the principal study planning directs the substance towards this group; what is not our case since the result indicates us two alternatives (classification in group 5 or no SGH classification),
- If we own reliable indications that the LD₅₀ will be located in range of group 5; or if other studies on animals or toxic effects observations noted at mankind give rise to motivated concerns for human health. We did not find such information in our bibliographical reference concerning *M. inermis* leaves,
- No mortality was observed by oral way testing up to values of group 4 nor was any significant clinical sign of toxicity in the test led at limit dose of 2000 mg/kg body weight not noted. In this scenario, the substance cannot be classified yet in group 5.

Through this analysis, we can say that our *M. inermis* leaves extract cannot be classified in SGH system as substance involving of acute toxicity with a LD₅₀ ranging between, 2000 mg/kg and 5,000 mg/kg. The estimated LD₅₀ of our extract is thus higher than 5,000 mg/kg. Extrapolating interpretation of this result to other scales toxic class, we note that Gosselin, Smith and Hodge scale (Anonymous, 1984; 2007a), our extract is slightly toxic (5,000 mg/kg < LD₅₀ 15,000 mg/kg) or almost non-toxic (LD₅₀ > 15,000 mg/kg); However, with Hodge and Sterner scale (Anonymous, 2007a), its toxicity index is either 5 that is, almost non-toxic (5,000 mg/ kg < LD₅₀

15,000 mg/kg) or 6 i.e. relatively harmless (LD₅₀ > 15,000 mg/kg). According to Kerharo et al. (1974), foliage of *M. inermis* was consumed by small ruminants, especially in dry zones and rarely by cattle. The food seems to be consistent with our results that showed no mortality in our extract up to a dose of 5,000 mg/kg body weight. It is therefore concluded that the high LD₅₀ obtained following i/p administration of the extract and lack of mortality when orally administered may be an indication that the aqueous leaf extract of *M. inermis* could be used with some degree of safety especially when consumed by oral route.

The qualitative study showed that all chemical groups identified in leaves extract of *M. inermis* are found in the traditional medicine preparation (leaves decoction). The extraction methods used in traditional medicine is qualitatively as effective as other extractions methods studied, namely infusion and extraction method by chloroform and methanol.

Sterols and triterpenes presence is in relation to that of saponosides. Indeed, saponosides are heterosides whose nucleus is either steroidal, or pentacyclic triterpenic (Gbeassor et al., 1989; Neuwinger, 1996; Koua, 1998; Bruneton, 1999; Kamanzi, 2002). Sterols produced ant rachitic D vitamin by irradiation. Some other vegetable sterols degradation could produce androgens steroids which could induce *Gambusia affinis* (fresh water fish) masculinisation under laboratory conditions, and polyterpenes are seem to be disinfectants, expectorants, diuretic and sedative.

Triterpenes and saponosides are also found at other species of *Mitragyna* such as *M. ciliata*, *M. stipulosa* and *M. rubrostipulata*, as well in leaves as in stems and roots barks (Frantisek et al., 1976; Nacoulma, 1996). Triterpenic saponosides being evoked in the majority of *Mitragyna* pharmacological effects, it is probable that antihypertensive properties allotted to *M. inermis* are related to this chemical compounds group. This work also shows that *Mitragyna inermis* leaves contain alkaloids like that was noted with other species of *Mitragyna* such

as *M. ciliata*, *M. stipulosa*, and *M. rubrostipulata*. These identified alkaloids have sedative, hallucinogens, ant malarial and anxiolytic properties in the bodies which contain them (Traoré et al., 2002; Asekun et al., 2006). Alkaloids action on central nervous system points out the action of reserpine; an alkaloid extracted from *Rauvolfia* (Apocynaceae) and endowed with antihypertensive properties (Dimo et al., 1999; Gharras et al., 1999; Datté et al., 2001; Ouédraogo et al., 2001; Traoré et al., 2002; Brock and Herzfeld 2006). Flavonoids are frequently diuretics and antispasmodics. They have P vitamin properties (resistance increasing and blood capillaries permeability reduction). Catechic tannins seem to be in connection with the scour use of *M. inermis* in wounds bandage and diarrhoea treatment by the fact of their astringent capacity (Ouédraogo et al., 2001; Traoré et al., 2002; N'Guessan, 2008). They are also used as vasodilator and haemostatic.

The absence of quinoid compounds in *M. inermis* leaves is characteristic to Rubiaceae family within which we finds plants known and very much used for their laxative and purgative properties in connection with the presence of anthracenic derivatives which are indeed heterosides with quinoid nucleus. *M. inermis* roots, barks and fruits being employed in some pharmacopoeias as laxative, purgative or haemolytic (Nacoulma, 1996; Koua, 1998; Ouédraogo et al., 2001), it is possible that these organs contain quinoid substances, contrary to leaves.

Conclusion

Toxicity study was carried out to situate *M. inermis* leaves tolerances limits. According to this study, the extracts used did not involve any mortality by predetermined dose method with a LD₅₀ estimated higher than 5000 mg/kg.

For the phytochemical screening, we highlighted the plant leaves richness in active compounds: sterols, polyphenols, flavonoids, catechic tannins, alkaloids and saponosides. This abundance in active elements confers to the plant, remarkable properties. This could justify its multiple therapeutic indications for which it is used in traditional medicine.

After phytochemical screening and toxicological studies of *M. inermis* leaves, it is advisable to evaluate pharmacological effects of this plant on antidiabetic activity; this in order to understand the virtues allotted to this vegetable species.

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