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Phytochemical and Toxicological Studies of an Extract of the Seeds of *Picralima Nitida* (Stapf) (Apocynaceae) and Its Pharmacological Effects on the Blood Pressure of Rabbit

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Abstract

The phytochemical screening of the seeds extracts of *Picralima nitida* has highlighted the presence of alkaloids and terpenes poly sterols in chloroform solutions, methanol and in the aqueous. Unlike chloroform and methanol solutions, the aqueous revealed the presence of saponins. An acute toxicity study in mice showed that the aqueous extract of *Picralima nitida* would be slightly toxic with a lethal dose (LD) 50 % of 9120.11 mg/kg of body weight (bw). This extract, in rabbits induced a dose-dependent hypotension for the doses between 3.10^{-6} g / kg bw and 2.10^{-5} g/kg bw with an effective dose 50% (ED50) equal to 4.07×10^{-6} g/kg bw. In the presence of atropine (6.10^{-9} g/kg), a competitive inhibitor of acetylcholine, the hypotensive effect of aqueous extract of *Picralima nitida* is reduced, confirming in this extract the presence of cholinomimetics substances of muscarinic type. The results therefore suggest the presence of cholinomimetics substances in the aqueous extract of *Picralima nitida* seed. These substances could be responsible for the hypotensive effect of this extract. The same extract did not induce diuresis in rats.

Keywords: *Picralima nitida*, Cholinomimetics substances, Atropine

1. Introduction

Picralima nitida called *Picralima macrocarpa* or *Picralima kleineana* or *Tabernaemontana nitida* (Omino, 2002) is a traditional medicinal plant widely used in Africa in the treatment of many diseases. It is a deciduous tree of about 20 meters high widespread in intertropical forested areas of Africa from Côte d'Ivoire (Ivory Coast) to Uganda through Zaire. This vegetable species is also found in mature secondary forest. The fruit is broadly abovoid, smooth and glabrous measuring about 15cm long and 10cm in diameter. Each fruit contains three flattened seeds embedded in pulp (Irvine, 1961; Keay et *al.*, 1964; Adjanohoun et *al.*, 1984, 1996; Shmelzer and Gurib-Fakim, 2008). *Picralima nitida* is used in traditional medicine for the treatment of diseases like malarial, typhoid fever, anemia, jaundice and dysmenorrheas (Jiofack et *al*., 2009). The Previous pharmacological studies of this plant extract showed that this plant possess sympathocostenic, antimalarial, antipsychotic and anaesthesic activities equivalent to that of cocaine (Perrot, 1944; Kerharo and Bouquet, 1950; Kapadia et *al*., 1993; Okunji et *al.*, 2005). It would also have antimicrobial, hypoglycemic and antidiarrheal properties (Fakeye et *al*., 2004; Nkere et *al*., 2005; Inya-Agha et *al*., 2006; Kouitcheu et *al*., 2006; Salihu et *al*., 2009). Since, some of activities of this substance have not been investigated scientifically. For this, the aim of this study is to provide a phytochemical screening, a toxicological study of extracts *Picralima nitida* seed and evaluate its pharmacological effects on blood pressure of the rabbit.

2. Materials and Methods

2.1 Plant Material

The seeds of *Picralima nitida* were purchased from an herbalist in yopougon market in the north of Abidjan (Ivory Coast). They were identified by an expert in botanic systematic, professor Ake-Assi of the national floristic centre of University Felix Houphouet-Boigny.

2.1.1 Preparation of Aqueous Extract of Picralima Nitida Seeds

The *Picralima nitida* seeds from dry fruits were ground with micro-crusher (Culatti, France). The powdered (5 g) of seed was diluted in 50 ml of boiled distilled water at 100 °C during 15 minutes. The infused is cooled and the solution obtained was filtered through of wattman paper n °1. The filtrate was frozen at -30 °C and lyophilized at -45 °C using a lyophilisator (Telstar, Spain). A brown colored powder was obtained. It is stored in the refrigerator at -5 °C in a sealed jar.

2.2 Animal Material

The animals used in our experiments, consist of mice (*Mus musculus*), rat (*Rattus norvegicus*) and rabbit (*Oryctolagus cuniculus*). The tests were performed only after that, rats were acclimated to the environment of the animal house of Biosciences. All procedures were approved by ethical committee of University Felix Houphouet-Boigny (Ivory Coast) and in accordance with the principles of scientific ethical committee of biology for use of laboratory animals for experimental tests (Aworet-Samseny *et al*., 2011).

2.2.1 Mice

The white mice (*Mus musculus*), from Swiss stump, weighing 25 to 30 g, were used for toxicological tests.

2.2.2 Rats

The Wistar rats (*Rattus norvegicus*) used for the diuretic activity weighed an average 150-200 g.

2.2.3 Rabbits

Only rabbits with a weight equal or greater than 1.45 kg were used. These animals were used for the measurement for arterial blood pressure.

2.3 Phytochemical Screening

The phytochemical screening was done using classic methods (Karumi et *al.* 2004; Bekro et *al*., 2007; Nene Bi *et al*., 2008; Farhat *et al.*, 2011; Soni and Sosa, 2013). Chemical compounds tested in all the extracts of *Picralima nitida* seeds were: saponosides, alkaloids, flavonoids, tannins, terpenes, sterols, phenols and quinones. The tests were based on the visual observation of color change or formation of a precipitate after specific reactions.

2.4 Oral Acute Toxicity

The healthy mice of both sexes were divided into 4 groups of 10 animals. Ten mice of equal numbers of male and female were used and each received a single oral-dose, using an intragastric cannula. Animals were kept overnight fasting prior to drug administration by gavage. The maximum dose volume administered did not exceed 2 ml/100 g body weight. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during 8 hours and daily thereafter, for a total of 14 consecutive days for behavioural changes and mortality.

2.5 Recording of Rabbit Blood Pressure

The experimental device used for recording of the blood pressure in the rabbit is based on the principle of the mercury manometer of Ludwig. The rabbits (1.45 kg) were anesthetized by intraperitoneal injection of ethyl–urethane 40 % dosed at 1 g/kg of body weight (bw). The saphenous vein from the leg was bared. This vein is intubated with a catheter attached to a syringe allowing the injection of different doses of the aqueous extract of *Picralima nitida* or atropine. For dose-response effect, the extract was administered alone. But to show the presence of cholinomimetic substance in the extract, atropine was administered before the extract. The rabbit carotid artery was exposed and intubated using a catheter connected to a U-tube manometer, which collects directly the intra-carotid pressure. It is the non-invasive method. This method measures the level of reference pressure in rabbits. Changes in the carotid pressure, transmitted to the mercury column of the device are recorded with a pen that translates the movements of mercury on the paper placed on a cylinder driven at constant speed by a motor.

2.6 Diuretic Activity

Twenty (20) male rats were used. These rats were divided into five equal groups (n = 4/group). After 18 hours of total fasting, they received 50 ml/kg of body weight (bw) of distilled water orally before treatment. To determine their influence on elimination of fluid overload, the excretion of sodium and potassium, the aqueous extract of *Picralima nitida* and the reference diuretic, the furosemide were administered to rats intraperitoneally. According Gallez *et al*. (1999), the intraperitoneal administration in rat presents high absorption rate compared to oral administration. This administration mode provides better bioavailability of the test substance in animals. Furosemide (Hoechst Houde, Germany) was purchased from the pharmacy. The animals in the control group were received intraperitoneally saline solution at the dose of 9 ‰. The rats were placed in metabolic cages (one in each cage) specially designed to separate urine and feces and maintained at room temperature. The urine volumes were determined after 24 hours. The concentrations of sodium, potassium in the urine and serum were determined using a flame photometer (optional ISE) with a multiparameter analyzer (Hitachi 902).

2.7 Statistical Analysis

The statistical analysis was performed using one-way analysis of variance (ANOVA) of the multiple test of comparison of Tukey-Kramer (GraphPad Prism software, version 4, San Diego, USA). The level of significance was determined in comparison with the control group. $p < 0.05$ was considered significant. All values are expressed as mean ± SEM.

3. Results

3.1 Phytochemical Screening of Picralima Notida Extract

The qualitative phytochemical analysis of chloroform, methanolic and aqueous extracts allowed to highlight the presence of sterols polyterpenes, alkaloids. In addition to these compounds, saponins have been identified in the aqueous (Table 1).

Tableau 1. Phytochemical screening of the extracts of *Picralima nitida* seeds

Chemical components	Chloroform extract	Methanolic extract	Aqueous extract
Quinones	-	-	-
Catechic tannins	-	-	-
Gallic tannins	-	-	-
Alkaloids	+	+	+
Sterols polyterpenes	+	+	+
Polyphenols	-	-	-
Flavonoids	-	-	-
Saponosides			+

Absence (-); Présence (+)

3.2 Toxicological Study

The oral administration of the aqueous extract of the seeds of *Picralima nitida* (*Pn*) at the doses ranging from 3408.9 mg/kg to 13620 mg/kg of body weight (bw) provokes a decrease of the motor activity in mice. These mice move firstly hardly. These dose-dependent phenomena occur after 20 minutes for doses less than 6810 mg/kg bw and after 15 minutes for the doses higher than 13620 mg/kg of the extract. Whatever at the dose of *Pn*, these animals are lying upon each other in a corner of the cage. The death of mice was observed for the doses equal or higher than 6810 mg/kg bw (Table 2) and the lethal dose 50% (LD50) was determined to be 9120.11 mg/kg for the graphic method (Figure 1) of Miller and Tainter, or 8852 mg/kg for calculation method of Karber and Behrens.

Tableau 2. Mortality in mice treated with the aqueous extract of *Picralima nitida*

Groups of mice	Dose of Pn (mg/kg bw)	Mortality (%)	Mortality (probit Unit)
I	3408.9	0	1.9
II	4539	0	1.90
III	6810	30	4.47
IV	13620	90	6.28

In this study, *Pn* caused the death in mice at the high doses like 6810 mg/kg bw.

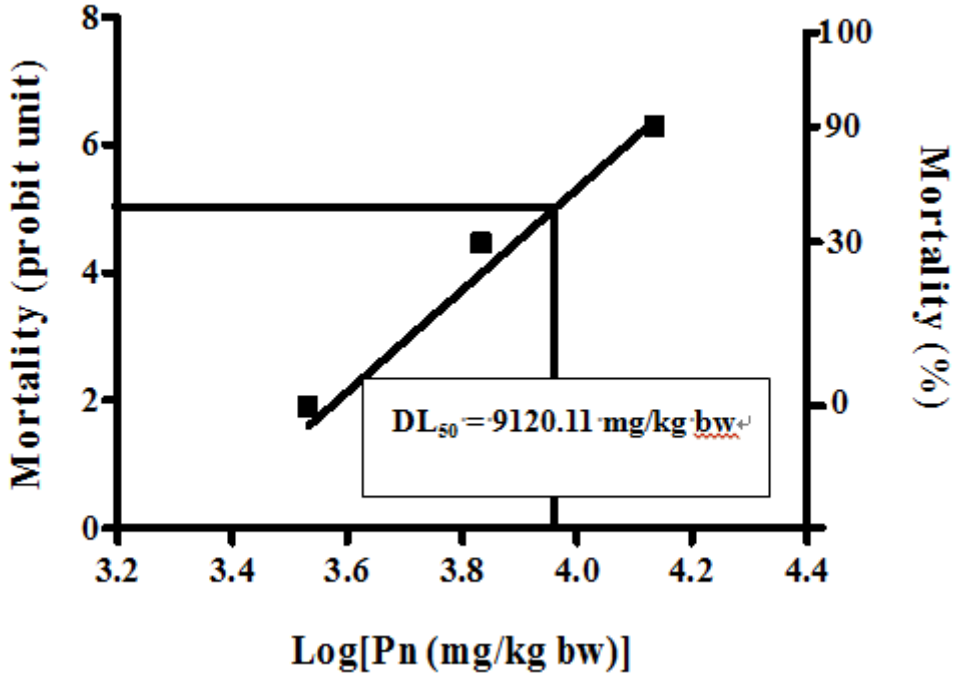

Figure 1. Oral acute toxicity study in mice treated with the aqueous extract of *Picralima nitida*. In presence of this extract considered slightly toxic, the LD50 was *9120.11 mg/kg bw.*

3.3 Effect of the Extract of Picralima Nitida on Blood Pressure in Rabbit

Figure 2A shows an original recording of dose-dependent hypotension induced by *Picralima nitida* aqueous extract (*Pn*). *Pn* for doses ranging from 3×10^{-6} g/kg to 2×10^{-5} g/kg bw, provokes a transient hypotension ranging from 20 mmHg to 36 mmHg. That corresponds to a decrease of the blood pressure of 39.83 ± 1.88 % and de 103.33 ± 6 % ($p < 0.001$).

In the presence of atropine (6×10^{-9}g/kg bw), an antagonist of muscarinic receptor of acetylcholine, the hypotension induced by *Pn* for doses ranging from 3.10^{-6} g/kg to 1.2×10^{-5} g/kg bw (Figure 2B) was reduced respectively from 22.22 ± 6 % ($p > 0,05$) to 55.56 ± 5 % ($p < 0,001$) (Figure 2C).

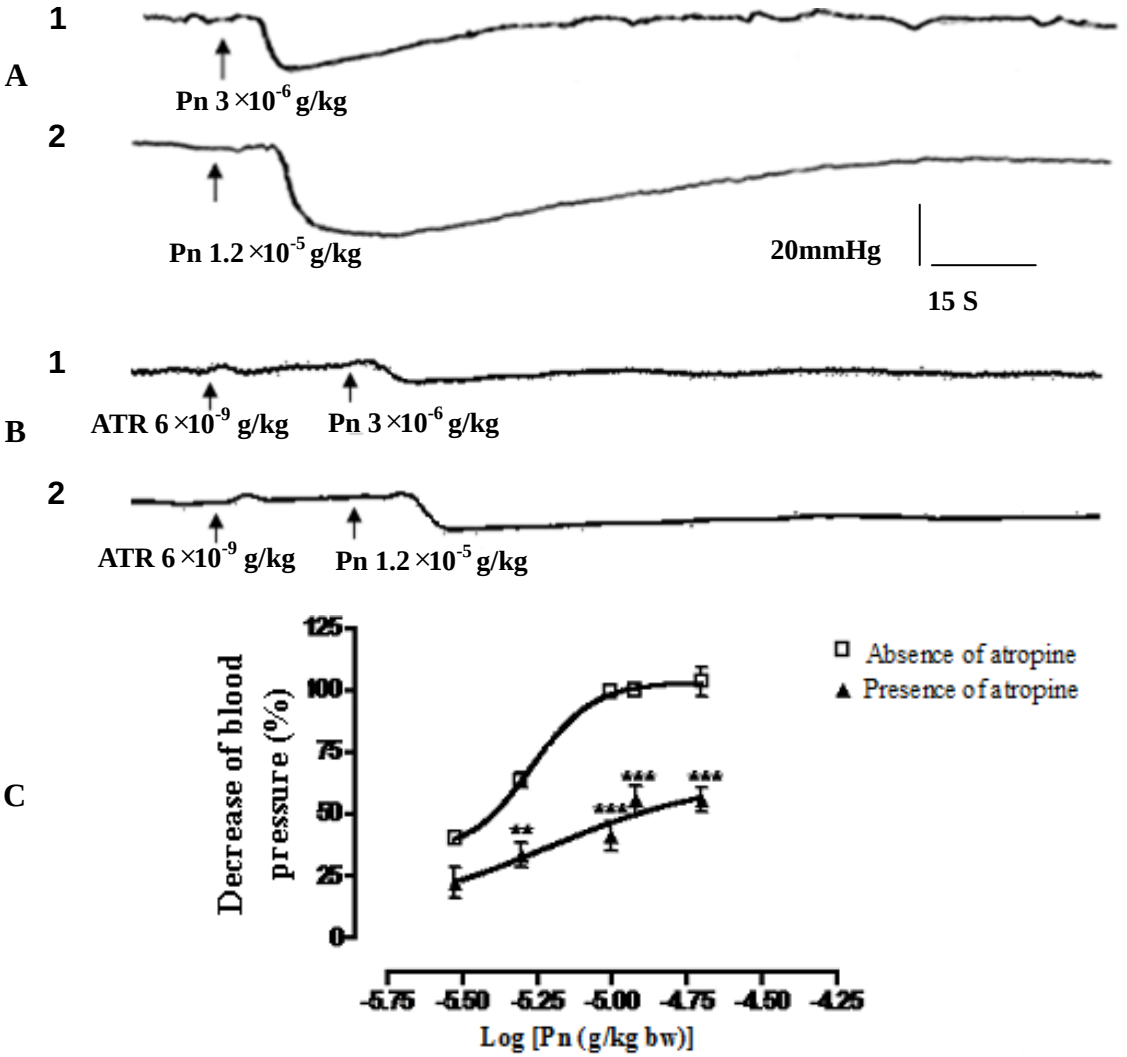

Figure 2. Dose-response effects of *Picralima nitida* on arterial blood pressure in rabbit. ***(A)*** *An original recording of the effect of Pn on the arterial blood pressure for doses ranging from* 3×10^{-6}*g/kg (**1**) to* 1.2×10^{-5}*g/kg bw (**2**). Pn causes a dose-dependent hypotension in rabbit.* ***(B)*** *The same doses of Pn are tested in presence of atropine (*6.10^{-9} *g/kg). This antagonist reduces hypotensive effect of Pn.* ***(C)*** *Curves showing a decrease of blood pressure in the rabbit in absence or presence of atropine.* Data are shown as means ± SEM, ***p<0.01;***p <0.001; n = 3.*

3.4 Diuretic Activities

Doses of *Pn* of 0.21 g/kg, of 0.42 g/kg and of 0.63 g/kg bw were used for this study. These doses did not modify significantly ($p > 0.05$) the urinary volumes measured in treated animals with *Pn* (18.58 ± 5.75 – 7.86 ± 4.21 ml/kg) compared to that of control (15.36 ± 3.93 ml/kg). For the same doses of this extract, the respective concentrations of urinary and plasmatic sodium and potassium were not very different ($p > 0.05$) compared with the control values.

4. Discussion

The results obtained in this study showed that the aqueous extract of the seeds of *Picralima nitida* contained alkaloids, sterols, polyterpenes and saponosides. These results confirm the previous study done by Perrot (1944), Kerharo and Bouquet (1950), Saxon (1973), Ansa-Asamoah et *al*. (1990), Kapadia et *al*. (1993), Duwiejua et *al*. (2002) and Okunji et *al*. (2005). These authors showed that this plant contained several alkaloids; the main ones were Akuammine and Akuammidine.

The acute toxicity study of *Picralima nitida* revealed a LD50 equal to 9120.11 mg/kg body weight (bw) which would render this plant slightly toxic according the classification of Diezy (1989) for substances. This extract is less toxic than the extracts of *Argania spinosa* (Dominique and Zoubida, 2005) and *Spondiathus preussii* (Ibo, 1977) which have lethal dose 50% respectively equal to 1.3 g/kg and 4.43 g/kg bw. On the other side, this extract would be more toxic than the extract of *Pilostigma reticulatum* which had a DL50 of 17 g/kg bw (Diallo and Diouf, 2000).

On blood pressure in rabbit, the aqueous extract of the seeds of *Picralima nitida* for doses ranging from 3×10^{-6} g/kg de PC to 1.2×10^{-5} g/kg bw, caused a dose-dependent hypotension. Similar hypotensive effects have been reported by Asgary *et al.* (2000) and Niazmand and Saberi (2010) on *Achillea millefolium* and Kitic *et al.* (2012) on *Sideritis raeseri*.

The hypotensive effect of the extract of the seeds of *Picralima nitida* reminds the effects of acetylcholine. The use of atropine, a competitive inhibitor of acetylcholine (Gerova et *al*., 2005; Hirota and McKay, 2006; de Azua *et al.* 2012; Jain *et al.*, 2012) reduced significantly the dose-dependent hypotension induced by *Pn* in rabbit. It indicates the presence of cholinomimetics substances in this crude extract. These results, comparable to those obtained by Dimo *et al.* (1999) with *Bidens pilosa* (Asteraceae), Abo *et al.* (2000) with *Mareya micrantha* (Euphorbiaceae) and Konan *et al.* (2006) with *Sesamum radiatum* (Pedaliaceae), Atsamo *et al.* (2013) on *Erythrina* senegalensis and Boua *et al.* (2013) on *Turraea heterophylla*, demonstrates the presence of cholinomimetics substances in the aqueous extract of *Picralima nitida*.

Indeed, acetylcholine by binding to its muscarinic type receptors on heart, could cause, negative inotropic and chronotropic effects (Felder, 1995; Bois *et al.*, 1999; Roffel *et al*., 2001; Racke and Matthiesen, 2004; Gerova *et al*., 2005; Hirota and McKay, 2006) and the vasorelaxation by releasing nitric oxide (NO) by endothelium (Furchgott and Zawadzki, 1980; Zapol *et al*., 1994; Ducrocq *et al*., 2001; Zhao *et al*., 2004; Lamblin *et al*., 2005; Collin and Levy, 2008; Jeanneret, Sanchez, Liaudet, 2011).

The hypotensive effects of *Pn* could be, probably the combined action of the effects of cardio-inhibitory and vasodilator substances.

If the hypotensive effect of the crude extract of *Picralima nitida* was demonstrated, this extract does not seem to have diuretic effect like the extracts of *Bidens pilosa* (Dimo *et al*., 1999) and *Bridelia ferruginea* (Nene Bi *et al*., 2012; 2013).

5. Conclusion

The phytochemical screening of the aqueous extract of the seeds of *Picralima nitida* revealed the presence of alkaloids, confirming the work of many authors. This extract considered slightly toxic, has hypotensive effects that would be due mainly to the cardio-inhibitors principles antagonized by atropine or not. This result is in agreement with the hypotensive effects of Akuammidine, one of the main alkaloids isolated from *Picralima nitida* seeds which advocates for the use of this plant in the treatment of hypertension.

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