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Research Article

Evaluation of *Picralima nitida* acute toxicity in the mouse

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ABSTRACT

Picralima nitida is a small tree used in a wide range by traditional medicine to cure various pathologies. This work was aimed to determine the toxicological parameters of the aqueous decoction of the plant seeds in order to control posology and prevent intoxication. The acute toxicity of seeds aqueous decoction from the plant was assessed after a process which consisted in giving orally, to mice, the crude decoction for increasing doses ranging from 600 to 3000 mg/kg of body weight (b.w.). The use of the herbal medicine, through oral route (or), at different doses, does not cause some clinical signs. The results made it possible to obtain the dose at bordering on solubility which squares with the tolerated maximal dose or TMD (3000 mg/kg/b.w./or). This toxicological parameter (tolerated maximal dose) is by far higher than 94.885 mg/kg b.w./or, the daily dose traditional healers recommended. Therefore, the dose prescribed by traditional healers is not toxic, justifying the use of the plant in traditional conditions of preparation and oral administration.

Key words: Tolerated maximal dose, Traditional medicine, Herbal medicine, Traditional healers

INTRODUCTION

Picralima nitida (Stapf) T. Durand et H. Durand (Apocynaceae), known in common language as Obéro or Demouain à grands fruits (French), is a small tree of 10 m in height. The cylindrical stem is much ramified. The plant products white latex. The leaves, simple, opposite, glabrous, are oblong-lanceolate to oblong-elleptic. Flowers, in terminal, white or yellow, are arranged in ombelliforme cyme. The fruit, a solitary berry or two connate berries, contains numerous seeds. The species, native of Africa, is found in dense rain forests: Eastern Côte d'Ivoire to South Nigeria and Angola. The plant is requested by the populations of developing countries, for its diverse therapeutic properties. In Côte d'Ivoire, Abbey and Krobou populations of Department of Agboville, use the plant for its antidiabetic and hypotensive effects[1,2,3]. According to Ouattara[4], the Dida of Divo, in the forest South of Côte d'Ivoire, use it as antipaludic. The Ibo in Southeast Nigeria produce medication from the plant for an aphrodisiac[5]. The pulp, mastered in alcohol, is used against pneumonia[5]. In Morocco, medicines are made from the plant to treat diabetes[6]. Populations of Cameroon use the plant for its antidiabetic effect[7]. In the same country, the fruit decoction is used to treat coughs or typhoid fever[8]. In Ghana, seeds are used to purge as an analgesic and chewed as a tonic and stimulant[9, 10]. The same authors indicated that the leaves decoction treats Guinea worm. In Gabon, populations of Pahouin tribe chewed the fruit to stave off hunger during long walks in the forest and used boiled seeds and bark with sugar against food poisoning or venereal diseases[11]. *Picralima nitida* is used by oral route in human therapeutic needs. However, in spite of this scientific knowledge, the use of the plant, in therapeutic purposes, in rural and traditional areas, has to put in front of obstacles, as that of doses used on the preparation and the administration of the medicinal receipts. This indistinctness constitutes a real problem of the traditional medicine[12]. The misunderstanding of the doses of extracts administered traditionally exposes the populations which use them at real risks of therapeutic accidents which can sometimes turn out tragic[13]. Present study was aimed to evaluate the acute toxicity of aqueous extract of *Picralima nitida* seeds used by oral route in adult mice of both sexes, and to determine the toxicological parameters which are imperative, to rationalize better its use.

MATERIALS AND METHODS

Traditional preparation and administration of herbal medicine

To look after the diabetes, the traditional healer recommends boiling, during 45 minutes, in a pan of water, four (04) handfuls of *Picralima nitida* seeds. After filtration, he is advised to adults, to drink a glass of the decoction (2 l of volume), three times a day, for a month of treatment. We evaluated vegetable quantity advised to adults for drinking, in the follow way:

$$M = \frac{\Sigma xi}{n}$$

M : Mean Mass of a handle seeds; n: Total number of handles

Xi : Mass of one handle; Σxi: Total mass of handles In accordance with this formula, we established the mean mass of handles seeds (table 1) and the mean volume of glasses (table 2).

Table 1: Mass of each handle of seeds

Handle of seeds	Mass (g)
1	156
2	149
3	135
4	158
5	160
6	157
7	144
8	138
9	146
10	157

Table 2: Volume of water in different glasses

Glasses	Administrated volume (ml)
1	170
2	180
3	191
4	201
5	162
6	185
7	175
8	178
9	182
10	176

Vegetable material

The drug (seeds of *Picralima nitida*), used in this study, were collected during rainy season in June 2013 within Port-Bouët II market of Yopougon in Abidjan (South of Côte d'Ivoire), freshly and rinsed. From the collected samples and specimens of the herbarium of the National Floristic Center, we identified the plant, by its scientific name. A voucher specimen (Abidjan, Côte d'Ivoire, March 3, 2013, N'guessan Koffi n° 400) was deposited in Herbarium of Botanic Laboratory, U.F.R. Biosciences (University Félix Houphouët-Boigny, Côte d'Ivoire).

Technical material for extract preparation

A Rotavapor was necessary to concentrate volume of decoction. A steam room at 60°C was used, to evaporate the filtrate of the drug decoction. An electronic balance of mark SHIMADZU AUX 320 Uni Bloc was employed, for the various weightings of crystals powders. The mice weight needed electric balance of mark TESTUT. The technical material also contained absorbent cotton and on Wattman paper, used as filters, a mortar and a porcelain pestle, to crush finely the obtained crystals, glass sterile jars and a refrigerator, for the preservation of the extract. Metal cages containing litters of shavings, spatulas, a nozzle of intubation, were necessary. All the technical material resulted from Laboratory of Pharmacognosy, U.F.R. Pharmaceutical and Biological Sciences (University Félix Houphouët-Boigny, Côte d'Ivoire).

Preparation of plant extract in Laboratory

One thousand (1000) grams of the drug (fresh seeds of *Picralima itida*) are rinsed then introduced in 4 liters of distilled water. The mixture, bulled during 45 minutes, was wrung in a neat cloth square, filtered successively twice on absorbent cotton and on Wattman 3 mm paper. The volume of the filtrate (2 l of decoction) obtained was concentrated with Rotavapor of type Büchi and evaporated in a drying oven at 60°C, during 2 days. The pulverized crystals made it possible to obtain fine powder (41 g) used for the experimentation. The total water extract, codified APN, is then kept in sterilized glass bowls, hermetically closed, in a fridge. The maximal concentration which corresponds to a concentration on limit of solubility of the extract is looked for by using 2 g of total extract in 20 ml of distilled water, that to say maximal concentration of 100 mg/ml. From this limit solution, successive dilutions are prepared in 1/2, 1/3, 1/4 and in 1/5. It allowed to obtain the respective concentrations of 50, 33.33, 25 and 20 mg/ml, which are used to lead the study of the acute toxicity.

Animals used

We used Healthy white mice (*Mus muculus*, Muridae) of SWISS race we obtained from Paster Institute of Adiopodoumé (Côte d'Ivoire). The animals (4-6 weeks old, 19-30 g of body weight, n=60 for all groups), with as many males as of females, were placed in ventilated metal cages containing litters of shavings which are regularly renewed. They were acclimatized to the conditions of the animal house, during 7 days before the treatment and fed with the granules produced by the Ivorian Compound Food Manufacturing Society (F.A.C.I.). We used tap water. Before treatment, the animals were deprived of food overnight. They were divided into 6 batches of 10, as follows:

batch 1-sample mice receiving distilled water (control group); batch 2-sample mice treated with herbal medicine at 100 mg/ml; batch 3-sample mice treated with herbal

medicine at 50 mg/ml; batch 4-sample mice treated with herbal medicine at 33.33 mg/ml; batch 5-sample mice treated with herbal medicine at 25 mg/ml; batch 6-sample mice treated with herbal medicine at 20 mg/ml.

Evaluation of toxicological parameters

This experiment on the acute toxicity was conducted with the aim of toxicological parameters determination such as the tolerated maximal dose or TMD, the lethal dose for 50% (LD_{50}) and the lethal dose for 100% (LD_{100}).

Determination of TMD and LD_{100}

Animals deprived of food overnight were dosed by oral gavage, using a nozzle of intubation, with 0.6 ml for physical 20 grams of aqueous solutions of plant extracts. Volumes from 0.57 to 0.90 ml were administered to animals, according to their physical weight. All mice were monitored continuously for 48 h after dosing for signs of toxicity. After the administration of the extract, animals are replaced in their metallic cages where they could have access to granules. They were observed immediately then every 30 minutes, during 8 hours, the first day and once a day, for 48 hours. During this period, clinical signs (agitation, dyspnea, lack of appetite, motor difficulty, and tendency in body weight) and the number of death animals are quoted.

Determination of the lethal dose for 50% (LD_{50})

The lethal dose for 50% was determined from formula of Karber and Berhens[14]:

$$LD_{50} = LD_{100} \frac{\Sigma (a \times b)}{n}$$

LD_{50}: Lethal Dose for 50%; LD_{100}: Lethal Dose for 100%; a: average of the sum of the deaths between two successive doses; b: difference between two successive doses; n: average of the number of animals used by batch.

RESULTS

Estimation of traditional dose (TD)

Mean mass of each handle of seeds (150 g) and mean volume of glass (180 ml) are determinate. From 1000 grams of the drug (fresh seeds of *Picralima itida*), we obtained 41 g of aqueous extract. The mass of extract due to a handle of seeds in the traditional preparation is Meh= (150 g / 1000 g) x 41 g = 6.15 grams. The mass of extract due to four (04) handles of seeds in the traditional preparation is Me4h = 6.15 g x 4 = 24.6 g. The concentration of *Picralima nitida* seeds in the traditional preparation is thus (C = Me4h / Vd = 24.6 / 2 = 12.30 g/l or 12.30 mg/ml), with Vd = 2 l (volume of the decoction in traditional preparation). The traditional healer recommends to the patient adult of approximately 70 kg, 3 glasses of the decoction, three times a day. In the applications of this medicinal recipe, the mass of extract absorbed every day (Mead) is = 3 x 180 x C = 3 x 180 x 12.30 = 6642 mg. The usual dose which he recommends (DQT) is = Mead x 1 kg / 70 kg = (6642 mg x 1) / 70 kg = 94.885 mg/kg p.c./or.

Determination of different experimental doses

Animals (male and female mice) were dosed by oral gavage, using 0.6 ml for 20 grams body weight of aqueous plant extracts. The different doses delivered were therefore 3000, 1500, 1000, 750 and 600 mg/kg b.w./or, by established batches (control and experimental groups) from respective concentrations of 100, 50, 33.33, 25 and 20 mg/ml (table 3). All animals were monitored continuously for 48 h after dosing for signs of toxicity.

Table 3: Level of mice mortality after administration of *Picralima nitida* seeds extract

Parameters	Level mortality by batch					
Established batches	1	2	3	4	5	6
Concentration (mg/ml)	0.6 ml/20g	100	50	33.33	25	20
Corresponding dose(mg/kg b.w./or)	30 ml/kg	3000	1500	1000	750	600
Number mice by batch	10	10	10	10	10	10
Number of death mice, 48 h after gavage of herbal medicine	0	0	0	0	0	0
Mortality (%)	0	0	0	0	0	0

Clinical signs quoted after the gavage of herbal medicine

The administration of *Picralima nitida* seeds extracts, through oral route, at different doses, from 600 to 3000 mg/kg b.w./or, caused any clinical signs, in the various established batches. A short agitation period was observed, during 3 minutes. However, any clinical sign such as lack of appetite, motor difficulty and dyspnea, was observed. Normal tendency in body weight was noted. In summary, any variation was observed on the experimental animals compared with the control group.

Gavage effect of herbal medicine on mice mortality

We observed any signs of toxicity during the acute investigation in control and experimental groups (table 3). *Picralima nitida* seeds extract, administered to doses of 600 to 3000 mg/kg b.w./or, caused the death of any animal, in the various established groups. There is no effect dose-response of the aqueous extract. The extract, administered by oral route, is deprived of toxicity, in the conditions of this study.

Evaluation of toxicological parameters

Visible sings of toxicity were not observed during experimental period in treated groups. The tolerated maximal dose or TMD was estimated at 3000 mg/kg b.w./or. In these conditions, the lethal dose for 50% or LD_{50} and the lethal dose for 100% or LD_{100}, are null.

DISSCUSSION

The investigations we conducted show that the plant extract exerts any mortality and toxicity effect on mice exposed to different doses of 600 to 3000 mg/kg b.w./or, in the various established batches. Our result is in accordance with previous report[15], looking at animals mortality. However, a significant difference is noted about the TMD value. The experiences we realized show that the seeds extract seems non harmless on mice, in 3000 mg/kg b.w./or. Instead of this value, Nana[16] reported a TMD value of 1200 mg/kg b.w./or, on rats. This difference as for the TMD value seems to be bound to the animals species employed for experiences. Our result does not confirm previous studies reported on the acute toxicity of *Picralima nitida* seeds. In Nigeria, *Picralima nitida* seeds showed, a low acute toxicity (LD_{50} value of 100 mg/kg): authors observed a reduction of motor activity and the death of some animals[12]. In the same country, intraperitonial administration of dichloromethane extract showed dose-dependent toxicity[17]. Others experiences reported abdominal pains, somnolence and agitation when rats received orally a low dose of *Picralima nitida* seeds extract. The high doses exert effect on eyes and mortality of rats within LD_{50} value of 14.5 g/kg body weight[18]. In a study of acute toxicity conducted in Tanzania, the seeds extract of *Picralima nitida* presented moderate toxicity, with a LD_{50} estimated of 16.3 microg / ml[19]. Folk medicine uses suggest a low toxicity of *Picralima nitida* seed extract. According to Chevalier[20], *Picralima nitida* seeds are used as an arrow poison in Ubangui (South-West of Central African Republic). In our study, the effects observed do not confirm it. The experiences we realized show that the seeds extract seems non harmless on mice, at 3000 mg/kg b.w./or. This difference as for the acute toxicity seems to be bound to the country of origin and to the various relative factors. With these few consistent results, *Picralima nitida* seeds extract can be taken with great precaution. As regards the study we conducted, the maximal concentration which corresponds to a concentration on limit of solubility represents the maximal tolerated dose (3000 mg/kg b.w./or). This TMD is widely superior to the dose of 94.885 mg/kg b.w./or, recommended by the traditional healer; it confirms the idea according to which the TMD is superior to the necessary doses to have pharmacological effects[21]. So, thanks to its DMT of 3000 mg/kg, the aqueous extract of *Picralima nitida* offers a considerable safety margin. Indeed, in the conditions of traditional use, it is almost impossible to reach the dose of 3000 mg/kg, with human being having 70 kg middleweight. So, the absence of toxicity of *Picralima nitida* reassures us as for the numerous uses of the plant seeds, in medicine of African tradition.

CONCLUSION

The results show that *Picralima nitida* seeds extract exerts any mortality and toxicity effect on mice, in the conditions of ours experiences. TMD (3000 mg/kg b.w./or) obtained is superior to folk doses evaluated (94.885 mg/kg b.w./or). The herbal medicine offers an interesting safety margin, what is reassuring as for its use in the treatment of various pathologies.

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