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RESEARCH ARTICLE

STUDY OF THE ACUTE TOXICITY OF THE AQUEOUS EXTRACT OF *TABERNANTHE IBOGA* (BAILL.) (APOCYNACEA) ON WISTAR RATS

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ABSTRACT

This study focused on the evaluation of *Tabernanthe iboga* (Baill.) acute toxicity. The lethal dose (LD_{50}) was determined on Thirty-five Wistar rats using BERHENS and KARBER's method. A total of seven batches of five animals including one control group were established and single doses of 392.1, 784, 71, 1000, 1570.5, 1750 and 2000 mg/kg were administered orally. The control group received distilled water. The results obtained allowed us to determine the LD_{50} at 1442.571 mg/kg of body weight. Therefore, *Tabernanthe iboga* could be classified as a low toxicity product.

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INTRODUCTION

Tabernanthe iboga is a small undergrowth shrub of dense forest in Central Africa. In Gabon, it is present throughout the territory. The leaves are simple and opposite with small flowers varying between yellow and white. The plant is widely consumed in Gabon by populations during traditional rites and in therapy as a neuro-muscular stimulant, antitoxic, anti-spasmodic (Raponda and Sillans, 1961). Numerous phytochemical studies have revealed the presence of alkaloids such as: *ibogaine, tabernanthin, ibogamine, ibolutein, noribogaine* (Lambert, 1902; Landrin, 1900; Delourme-Houdé, 1944; Goutarel and 1957; Asta, 2008). Pharmacological studies have shown that certain Tabernanthe iboga alkaloids have effects on different addictions: addiction to opiates, alcohol and cocaine (Kenneth, 1999; Mash and *al*., 2001; Dao-Yao and *al*., 2005) and on rat vas deferens (Valette an *al*., 1977). In addition, studies on aqueous extracts have shown a vasodilator effect on the aortic smooth muscle (Bourobou and *al*., 2014). In terms of toxicity, studies have been rather oriented towards alkaloids such as *ibogaine* whose LD_{50} is 263 mg / kg in the oral mouse (Asta and *al*., 2008) and *noribogaine* whose LD_{50} is 630 mg / kg in mice orally (Asta and *al*., 2008). Raponda and Sillans (1961) have observed that, in large doses, iboga makes you lose your mind, causes hallucinations and sometimes death. Knowing the wide use of this plant in Gabon and the lack of information relating to the acute toxicity of total extracts of Tabernanthe iboga, it was opportune for us to assess the short-term toxicity of this plant species. The present study consists in determining the acute toxicity of the bark of the roots of Tabernanthe iboga after a single administration of the aqueous extract by the oral route.

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MATERIAL AND METHOD

PLANT MATERIAL

The roots of *Tabernanthe iboga* from the stalls of a traditional healer were identified by botanists from the National Herbarium of Gabon from samples No. 927 (February 19, 2006) and No. 1636 (July 4, 2006).

Animal material: Thirty five adult *Wistar* rats weighing between 140 and 300g from the pet store of the Institute of Traditional Pharmacopoeia and Medicine (Iphametra) located at the Sibang arboretum in Gabon were divided into 7 batches of 5 rats.

Methods

Preparation of the aqueous extract of Tabernanthe iboga: The root bark was dried at room temperature and then ground. 100 g of powder was macerated in water for 48 hours. After filtration, the filtrate was frozen for 24 hours and then lyophilized for 72 hours in a freeze dryer (CHRIST BETA 1-8 K BIOBLOCK SCIENTIFIC USA). A quantity of 6.02 g of raw aqueous extract of the root bark was obtained and stored in a desiccant bell. The lyophilized extracts enabled us to carry out the various tests.

Preparation of doses to be tested: The preparation of the doses to be administered was carried out according to the protocol of Konkon et al, 2006. We first sought the maximum concentration which corresponds to the limit of solubility of the product in water. The maximum concentration obtained is 52.35 mg / ml equivalent to a dose of 1570.5 mg / kg of body weight. From the limiting solution, we made successive 1/2 and 1/4 dilutions which gave respective concentrations of 26.175 mg / ml (dose of 784, 71 mg / kg) and 13.09 mg / ml (dose of 392.1 mg / kg). For a better evaluation of the $LD_{50,}$ we have fixed in addition 3 intermediates doses: 1000 mg / kg, 1750 mg / kg and 2000mg / kg.

Toxicity test: Acute toxicity is materialized by Lethal Dose 50 (LD_{50}) which corresponds to the dose which causes 50% mortality in rats in a batch. The animals were fasted for 24 hours. The different solutions were administered orally using a rigid probe with an olive tip in the following order:

The control batch was treated with distilled water

Lot I dose of 392.1 mg / kg,
lot II dose of 784.71 mg / kg,
lot III dose of 1000 mg / kg,
batch IV dose of 1570.5 mg / kg,
batch V dose of 1750 mg / kg and
batch VI dose of 2000mg / kg.

After the administration of the aqueous extract, the animals were observed for 4 h on the first day and the following days for 14 days. During this observation phase, we noted symptomatic disturbances and the number of deaths.

Expression of results

We adopt the method of KABBER and BERHENS for the calculation of the LD_{50}.

$DL_{50} = DL_{100} - (\Sigma ab / n)$
LD_{50} = Dose giving 50% of deaths
DL_{100} = Dose giving 100% death
a = average of deaths between two successive doses
b = difference between two successive doses
n = average of animals used

RESULTS

The administration by gavage of the crude aqueous extract of *Tabernanthe iboga* to the different batches of rats at variable concentrations showed several intermittent clinical signs (tremors, loss of muscular coordination, difficulty breathing, convulsions, muscular spasms, short-term paralysis) sometimes followed by the death of animals (Table 1).
The DL_{50} value obtained is 1442.571 mg / kg. The LD_0 and LD_{100} were estimated at 784, 71 mg / kg and 2000 mg / kg respectively (Table 2).

DISCUSSION

Few studies have been published on the total extract of Tabernanthe iboga. For the first time, in 1867, Griffon du Bellay studied the roots of *Tabernanthe iboga.* He sent them to Europe and signaled that these, tonics in high doses, are an exciter of the nervous system. *Iboga* owes its properties to a special alkaloid *ibogaine*, the main activity of which relates to the central nervous system and the cardiovascular system (Landrin, 1900, Delourme-Houdé, 1944; Mash and al., 2001).
The symptoms observed in our study are comparable to those observed in studies carried out on ibogaine. *Tabernanthe iboga* therefore causes tremors at low doses (392.1 mg / kg) orally. As the doses are increased, the tremors become intense. Similar results have been observed in rats by French after injection of 40 mg / kg of *ibogaine* intraperitoneally (i.p.), they also observe fine tremor that persists for 2 hours (French et al., 1996). Hearn & Molliver (1993) note the appearance of ataxia and very rapid and intense tremors of the head and trunk at doses close to 100 mg / kg in rats.

From 784, 71 mg / kg, we observed a loss of motor coordination which increases and intensifies at high doses (1570.5; 1750 and 2000 mg / kg) with the appearance of intermittent convulsions, propulsion far from ground and short-term paralysis. This is in line with the results observed by O'Hearn and Molliver, who in a study aimed at evaluating the toxicity of *ibogaine* in rats, at a dose of 100 mg / kg per i.p. note the appearance of a great motor incoordination and for some, jerky extension movements of the limbs propelling them very far from the ground. From 784, 71 mg / kg, we observed a loss of motor coordination which increases and intensifies at high doses (1570.5; 1750 and 2000 mg / kg) with the appearance of intermittent convulsions, propulsion far from ground and short-term paralysis. This is in line with the results observed by O'Hearn and Molliver, who in a study aimed at evaluating the toxicity of ibogaine in rats, at a dose of 100 mg / kg per i.p. note the appearance of a great motor incoordination and for some, jerky extension movements of the limbs propelling them very far from the ground. The authors also observe hypotonia of the trunk, flaccid hind legs, the animals lying down. Spontaneous locomotor activity is restored the next day. The symptoms observed above are similar to those in our study.

Some clinical studies have shown in humans the appearance of a slight motor incoordination within 1 to 2 hours after ingestion of ibogaine, at the same time as the onset of nausea and / or vomiting Naranjo (1969) , Lotsof (1995) and Luciano (1998). Fernandez et al (2001) have estimated high doses of ibogaine at approximately 35 mg / kg in humans. At these doses, the primary effects are hallucinations, possibly accompanied by unpleasant side effects. The clinical picture presented by people who ingested these high doses is comparable to that described by Lambert (1901) in animals, namely: convulsions, paralysis and respiratory arrest (Popik et al., 1998). At the highest dose (2000 mg / kg) of iboga extract, the following effects were observed which coincide with those

Table 1. Symptoms and mortality of rats according to the dose administered

Different lots	Number of rats	Doses administered (mg / kg)	symptoms	Number of dead rats	% mortality
Witness Lot	5	Distilled water	No signs	0	0
I	5	392,1	Light tremors	0	0
II	5	784,71	Tremor, difficulty breathing, loss of motor coordination	0	0
III	5	1000	Tremor, difficulty breathing, loss of motor coordination, seizures	1	20
IV	5	1570,5	Tremor, difficulty breathing, loss of motor coordination, muscle spasms, short-term paralysis, seizures	3	60
V	5	1750	Tremor, difficulty breathing, loss of motor coordination, muscle spasms, short-term paralysis, seizures	3	60
VI	5	2000	Breathing difficulties, loss of motor coordination, muscle spasms, paralysis, seizures	5	100

Table 2. Determination of the LD_{50} in rats according to KABBER and BERHENS

	Lot I	Lot II	Lot III	Lot IV	Lot V
Dose (mg / kg)	784,71	1000	1570,5	1750	2000
Number of animals	5	5	5	5	5
Number of deaths	0	1	3	3	5
Difference in successive doses (b)	251,29	570,5	179,5	250	-
Average death between doses (a)	0,5	2	3	4	-
(a) x (b)	107,645	1141	538,5	1000	-

DL_{50}= DL_{100} – (Σab/n) DL_{50}= 1442,571 mg/kg

Table 3. Toxicity class of aqueous maceration studied according to the Hodge and Sterner toxicity scale (1943)

Toxicity class	LD50 (rat, mouse) mg / kg	Doses for a child 12.5 kg
Extremely toxic	<18 mg	Taste it
Very toxic	1 to 50 g	500 mg (swallowing a sip)
Moderately toxic	50 to 500	Swallowing a teaspoon
Slightly toxic	500 to 5,000	60 g (the fact of eat an egg cup)
Practically non-toxic	5,000 to 15,000	180g
Relatively safe	> 15,000	> 180 g

observed in humans after consumption of ibogaine in high doses: difficulty breathing, loss of motor coordination, muscle spasms, paralysis, convulsions followed by the death of animals. Based on these observations, ibogaine could be involved in the onset of these symptoms. However, the LD_{50} value of 1442.571 mg / kg, between 500 and 5000mg / kg, allows Tabernanthe iboga to be classified in the category of slightly toxic products (toxicity class of Hodge and Sterner). Studies of the acute toxicity of ibogaine yield LD50 values which vary depending on the animal and the route of administration. In guinea pigs, the LD50 is 82 mg / kg per ip, in rats, it is 327 mg / kg orally and 145 mg / kg intraperitoneally (Hough et al, 1996; Delourme-Houdé, 1946) and 175 mg / kg per intraperitoneal view in mice (Alper, 2001). Ibogaine toxicity studies have shown that oral LD50 in mice is estimated to be 263 mg / kg (Asta et al, 2008), which classifies ibogaine as moderately toxic . However, it emerges from our work that iboga is slightly toxic. This low toxicity observed in iboga compared to ibogaine which is a molecule is probably linked to the presence of other chemical compounds which interact. (Delourme-Houdé, 1944; Bruneton, 1999).

Conclusion

All the effects observed in animals as well as in humans (tremors, prolonged and often painful loss of muscle coordination, convulsions) show that the iboga extract acts mainly on the central nervous system. Most of the studies on Tabernanthe iboga have been focused mainly on ibogaine. However, at high doses, ibogaine is toxic to the nervous system because it destroys the neurons of the cerebellum (O'Hearn & Molliver, 1993; Scallet et al, 1996). In our study, we demonstrated that iboga belongs to the category of low-toxic products (LD50 of 1442.571 mg / kg), which is why it is recommended to use Tabernanthe iboga in a moderate way. Sub-chronic and chronic toxicity studies combined with histopathological analyzes should be performed to assess the effects of the aqueous extract on the organs.

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