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Acute toxicity effects of dichloromethane fraction of ethanol extract of stem bark of *Piliostigma reticulatum* on rats

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The aim of this study was to investigate the oral acute toxicity effects of dichloromethane fraction of ethanol extract of stem bark of *Piliostigma reticulatum* on rats using conventional methods. The rats were divided in nine groups. The control group received normal saline. The others groups received the order of doses of 800, 1000, 2000, 3000, 4000, 5000, 6000 and 7000 mg/kg body weight. However, the rats showed signs of immobility with the dichloromethane fraction at doses of 5000 mg/kg body weight and mortality at doses of 2000 mg/kg body weight. The LD50 was 3000 mg/kg body weight and the quotient LD5/LD95 was 0.17. This value shows that the toxic dose and the therapeutic dose were not distant. Phytochemical screening revealed the presence of tannins, flavonoids, polyphenols and reducing sugars in the stem bark of *P. reticulatum*. These results suggest that dichloromethane fraction of the ethanol extract of stem bark of *P. reticulatum* could be used with some degree of safety especially by oral route.

Key words: Acute, toxicity, stem, bark, *Piliostigma reticulatum*, rats.

INTRODUCTION

Hundreds of years ago, plants were known to play mainly nutritional roles. Today they play an additional important function in treatment of diverse pathologies. Plants, although pharmacologically active, are used without knowledge of toxicological parameters. For this reason, the World Health Organisation approved the use of herbal products for national policies and drug regulatory measures in order to strengthen research and evaluation of the toxicity of these products (Saxena, 2001). Thus, the toxicity of certain plants has been shown in many studies. For example, the acute toxicity of *Anogeissus leiocarpus, Daphnia magna* and *Cansjera rheedii* has

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been studied respectively by Agaie et al. (2007), Altindag et al. (2008) and Mounnissamy et al. (2010).

Piliostigma reticulatum which is the object of our study is traditionally used in Côte d'Ivoire for diarrhoea including bacterial diarrhoea (Yelemou et al., 2007; Dosso et al., 2012). Thus Babajide et al. (2008) have shown that the piliostigmol (6-C-methyl-2-p-hydroxyphenyloxychromonol), a substance isolated in *P. reticulatum,* inhibited *Escherichia coli* with a MIC equal to 2.57 µg/mL. In our previous study, results showed that an ethanol extract of the stem bark of *P. reticulatum* significantly reduced the gastrointestinal transit, the number, volume and weight of faeces of rats (Dosso et al., 2012). Also, the extract was not toxic in rats. In the present study, the aim of the current investigation was to determinate the toxic effects of the dichloromethane fraction of a crude ethanol extract of the stem bark of *P. reticulatum* on rats as well as to screen its phytochemical constituents.

MATERIALS AND METHODS

Plant collection

Stem barks of *P. reticulatum* (DC.) Horscht (Ceasalpiniaceae) were collected in Abidjan (South region of Côte d'Ivoire) in October 2007. The plant was identified and authenticated by Pr AKE-ASSI Laurent. A voucher specimen (No. 18033) of the plant has been deposited in the herbarium of the National Centre of Floristic of University of Cocody-Abidjan.

Preparation of dichloromethane fraction

Stem barks of *P. reticulatum* were washed with distilled water, cleaned, cut into smaller pieces and kept at room temperature for two weeks. Then they were ground into a fine powder. The powder (100 g) was extracted with two liters of a solution of ethanol (96%) / water (80:20) for 24 h under constant stirring (this operation was repeated twice).

The extract was filtered twice through cotton wool, then through Whattman filter paper (No 1). The filtrate was evaporated to dryness using a rotavapor (Buchi R110/NKE6540/2) at 45°C, and dried under reduced pressure. Percentage yield was found to be 13.6%.

After successive liquid-liquid fractionations, five fractions (heptane, dicloromethane, ethyle acetate, butanol and water) were obtained from the crude ethanol extract (Harborn, 1984; Samsam-Shariat, 1992).

Animals

Healthy young adult albino Wistar rats (weighing 150-200 g) of both sexes that were provided by UFR Biosciences (University of Cocody-Abidjan, Côte d'Ivoire) were housed in standard metal cages. They were kept under standard laboratory temperature conditions one week before the experiments for acclimation. The animals were fed with a diet of commercial pellets (Ivograin®, Abidjan, Côte d'Ivoire) and were given water *ad libitum*. They were deprived of food for at least 18 h prior to experiments but allowed free access to drinking water. The equipment usage and handling and sacrificing of the animals were in accordance with the European Council legislation 87/609/EEC for the protection of experimental animals (Mitjans, 2008).

Acute toxicity studies

Fifty forth albino rats were divided into nine groups of six rats each and were given graded doses (800, 1000, 2000, 3000, 4000, 5000, 6000 and 7000 mg/kg body weight) of the fraction by oral route. The rats were observed for signs of toxicity and death over a period of 72 h as described by Lorke (1983). The first group received single oral dose of 2 ml of normal saline through the same route. The LD_5, LD_{50} and LD_{95} of the fraction were calculated using the arithmetic method of Karber as modified by Aliu and Nwude (1982).

Phytochemical analysis of the fraction

The dichloromethane fraction was screened for the presence of tannins, flavonoids, alkaloids, sterols, saponins, polyphenols, polyterpenes and anthraquinones. Detection of these constituents was performed according to Bekro et al. (2007).

RESULTS

Phytochemical analysis of the fraction

Phytochemical screening tests of dichloromethane fraction for various constituents revealed the presence of major components such as tannins, polyphenols and flavonoids. Reducing sugars were present, and anthraquinones, alkaloids, coumarins, polyterpenes and sterols were absent.

Acute toxicity studies

The results in the Table 1 show that with the dichloromethane fraction at doses of 800 to 4000 mg/kg body weight, the survival animals are mobile. At the dose of 5000 mg/kg body weight, we observed the immobility of rats. Also the animals began to die at dose of 2000 mg/kg body weight. At dose of 1000 mg/kg body weight, we had 0% of mortality. The LD_{50} (50% of mortality) is 3000 mg/kg body weight. Also, at the dose of 7000 mg/kg body weight, 100% of animals did not survive with dichloromethane fraction (Tables 1 and 2). The DL_5 and DL_{95} are respectively 1118 and 6425 mg/kg body weight (Figure 1). The quotient DL_5 / DL_{95} reports 0.17. This result is far from 1.

DISCUSSION

The study of the acute toxicity of dichloromethane fraction showed that the LD_{50} is equal to 3000 mg/mL body weight. Animals showed toxicity signs in a dose-dependent manner. Also, at a dose of 7000 mg/mL, there were no survivors. The results obtained with the dichloromethane fraction, are consistent with those of Diallo and

Table 1. Acute toxicity signs observed in rats after treatment with dichloromethane fraction.

Dose (mg/kg body weight)	Acute toxicity signs	
	mobility	Death
800	+	_
1000	+	_
2000	+	+
3000	+	+
4000	+	+
5000	_	+
6000	_	+
7000	_	+

Table 2. Percentage of mortality in rats treated with dichloromethane fraction.

Group	Number of animals	Dose (mg/kg body weight)	Number of death	Mortality (%)
1	6	800	0	0
2	6	1000	0	0
3	6	2000	2	33.33
4	6	3000	3	50
5	6	4000	4	66.67
6	6	5000	4	66.67
7	6	6000	5	83.33
8	6	7000	6	100

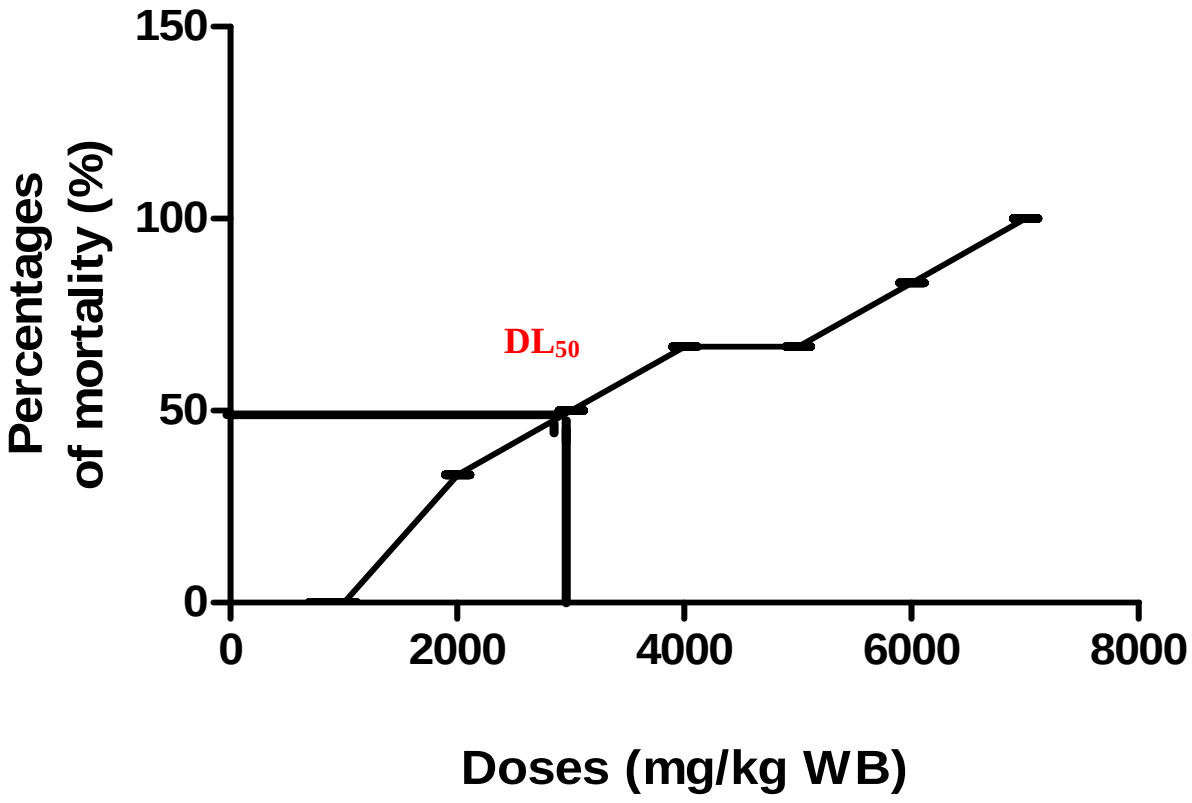

Figure 1. Evolution of mortality percentage in rats treated with dichloromethane fraction.

Diouf (2002) which showed that the LD_{50} of the aqueous extract of *Piliostigma reticulatum* leaves in rats was 1700 mg/mL body weight. The leaves of this plant therefore center more toxic substances in the leaves than stem bark. The LD_{50} obtained with our active fraction is higher than that obtained with the aqueous extract of the leaves. Agaie et al. (2007) studied the acute toxicity of aqueous extract of leaves of *Anogeissus leiocarpus* rats and was near 1200 mg/kg body weight like LD_{50}. This plant is more toxic than that used in our studies with a low LD_{50}. The study of the toxicological properties of the latex of *Calotropis procera* yielded approximately a LD_{50} like 2611.75 mg/kg body weight (Lohoues et al., 2006). The latex of *Calotropis procera* focuses more toxic substances than the dichloromethane fraction.

The quotient DL_5 / LD_{95} (0.17) is far from 1. These results indicate that the use of this fraction is not safe in its therapeutic use with the understanding that the therapeutic dose is not distinct from its toxic dose (Tamboura et al., 2005). These results are contrary to those obtained by Lohoues et al. (2006). According to these authors, the DL_5 / DL_{95} quotient in the study of the acute toxicity of the latex of *C. procera* was 0.71 - 1. The therapeutic dose of this product is distinct from the toxic dose. The latex of this plant is probably non toxic.

After administration of the fraction of plant, immobility and death of animals are observed at high doses. These clinical manifestations had been also reported by Lohoues et al. (2006) in the study of the acute toxicity of the latex of *C. procera* in Côte d'Ivoire.

The phytochemical screening of dichloromethane fraction showed the presence of flavonoids, tannins, polyphenols and reducing sugars. Our tests have not revealed the presence of alkaloids, quinones, coumarins, saponins, sterols and polyterpenes. Phytochemical studies by Kubmarawa et al. (2007) on the aqueous extract of the roots of *P. reticulatum* showed the presence of chemical compounds such as saponins, tannins and flavonoids. Contrastingly, chemical compounds in the same plant could be explained by the different organs used in the studies or the difference of natural environments. Thus, we observed a variation of secondary metabolites in the same plant according the living environment (Sofowora, 1996).

The active compounds observed in the stem bark of *P. reticulatum* could explain the toxicity signs in rats. Thus, when in excess in the body, certain chemical compounds may exceed their therapeutic activities, incite some malfunctions or lethal disorders (Lohoues et al., 2006). For example, astringent tannins have a role in reducing foods in animals (Alldredge, 1993; Agaie et al., 2007). This causes weakness in animals leading to immobility. Polyphenols are endowed with surfactant and hemolytic properties (Lohoues et al., 2006).

Thus, according to the same authors, compounds that are swallowed or even inhaled, are known to cause, among others, digestive tract burns, cyanosis, hypoxia and seizures.

Schultz and Riggin in 1985 stated in this regard that the phenol poisoning lead to death by acute respiratory failure. These could have caused immobility and death of the animals in our study.

Conclusion

Our results show that the stem bark of *P. reticulatum* contains toxic natural substances. These could be due to chemical constituents observed in dichloromethane fraction like tannins, flavonoids, reducing sugars and polyphenols.

It will be necessary to eliminate the probable toxic substances in dichloromethane fraction. Thus, the fraction could be used to treat diarrhea without risk. Also, the use of aerial parts of the plant could represent an alternative to the utilization of its roots, therefore limiting the biodiversity degradation.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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