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Phytochemical and acute toxicity study of aqueous extract of *Bambusa vulgaris* leaves on Wistar rats

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Abstract

Background: *Bambusa vulgaris* (Poaceae) is widely used in the traditional pharmacopoeia to treat various pathologies like typhoid fever, diabetes, hypertension and malaria. **Methods**: The present study has demonstrated the different phytochemicals and the acute toxicological study of the aqueous extract of *Bambusa vulgaris* leaves (EABV). For the phytochemical study, determination of secondary metabolites by tube tests was used. The acute toxicity of EABV was performed according to the guidelines of OECD 423. The doses of 300 mg/kg and 2,000 mg/kg were administered by intraperitoneal route to rats. Clinical signs, mortality, body weight, relative weight of organs, hematological and biochemical were noted. **Results:** *Bambusa vulgaris* leaves contain flavonoids, polyphenols, catechin tannins, saponins, and alkaloids. The toxicological study of EABV reveals that the LD_{50} is comprised between 500 mg/kg and 2,000 mg/kg. The animals exhibited several behavioral disorders. The body mass gain and the relative mass of the organs have not been modified. However, the single injection of EABV induces a variation of some hematological and biochemical parameters of the animals at the end of the experiment. **Conclusion:** *Bambusa vulgaris* leaves contain polyphenols, flavonoids, alkaloids, catechic tannins and saponosides. It's weakly toxic when injected intraperitoneally and affects hematological and liver parameters.

Keywords: *Bambusa vulgaris, Aqueous extract, Phytochemical study, Acute toxicity, Intraperitoneal route*

1. Introduction

For old age, medicinal plants have been widely used as a first alternative in the treatment of many pathologies. According to WHO statistics (WHO, 2008), more than half of the world's population uses medicinal plants for treatment because they are an integral part of the civilization of peoples. In addition, the high cost of care

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justifies the use of plants in primary health care (Hudaib *et al*., 2008). This great interest in herbal medicine has prompted scientists to conduct several studies to provide tangible evidences on the beneficial effects of these plants. Among these plants you have *Carica papaya* (Caricaceae) whose nephroprotective effect of the seeds has been proven (Kanadi *et al*., 2019). Works conducted by Nafiu *et al.* (2018) on the leaves of *Paullinia pinnata* (Sapindaceae) showed the antidiabetic, hypolipidemic and antioxidant properties of this plant. The multiple virtues of medicinal plants justify their use on a large scale in the traditional pharmacopoeia. Their properties are attributable to the different chemical compounds they contain. However, administration of the plants may be lethal or may cause immediate or late damage to the exposed subject. In addition, the intensity of these effects is a function of the plant used and the physiological state of the individual. Therefore, a toxicological study of plants is essential to better control their use in the treatment of diseases.

Bambusa vulgaris is a plant of the family Poaceae. This species is widely used to treat various diseases including typhoid fever, malaria, diabetes and high blood pressure (Hessavi *et al*., 2019). In the traditional pharmacopoeia, the decoction of *Bambusa vulgaris* leaves is used to treat high blood pressure (Kimpouni *et al*., 2018). Its use in the management of certain diseases has led to several studies. Thus, the hypoglycemic effects (Senthilkumar *et al*., 2011), antimicrobials (Owolabi and Lajide, 2015) and its toxicity (Yakubu *et al*., 2009) have been revealed. The present study consists of determining the different phytochemical substances and assessing the acute toxicity of the aqueous extract of *Bambusa vulgaris* (EABV) leaves by intraperitoneal route in rat.

2. Materials and methods

2.1. Animals

Female albino rats (*Rattus norvegicus* strain wistar) weighing between 120 g and 150g were used for the acute toxicity study. These animals were fed *ad libidum* and raised following the 12 h / 12 light- darkness cycle at the vivarium of École Normale Supérieure (Abidjan-Côte d'Ivoire). All animals were acclimated for seven days prior to experimentation.

2.2. Plant material and extraction method

The *Bambusa vulgaris* leaves were collected in Bingerville (Abidjan / Côte d'Ivoire) in February 2017. The sample was authenticated by Late ASSI Jean and deposited in the herbarium (UCJ006786) of the National Floristic Center (Felix HOUPHOUET-BOIGNY University, Abidjan, Côte d'Ivoire). The fresh leaves are washed, dried in the shade and sprayed in a Reich type electric shredder. 100 g of leaves powder was boiled in 1 L of distilled water for 10 min. After cooling, the decoction is filtered on hydrophilic cotton and on wattman paper n°1. The filtrate is then dried in an oven (45 °C) of the Memmert D-91126 Schwabach FRG (Germany) type. The powder obtained is the aqueous extract of *Bambusa vulgaris* leaves (EABV).

2.3. Output extraction

The output (*R*) is determined by the ratio of the mass of the dry extract (*M*) obtained on the test portion (*PE*) of the vegetable powder. It is expressed in percentage.

$$R = \frac{M}{PE} \times 100$$

R: output (%); *M*: mass of the dry extract (g); and *PE*: test sample of plant material (g).

2.4. Phytochemical analysis

The phytochemical compounds of the aqueous EABV leaves were identified by the reactions in tube (Bagre *et al*., 2007).

2.5. Acute toxicity test

This study was conducted according to the guideline 423 of the Organization for Economic Cooperation and Development (OECD, 2001). The animals are distributed in cages labeled in three groups of three rats. Groups 1 and 2 received intraperitoneally (*i.p*) a single doses of 300 mg / kg and 2,000 mg / kg. The third group (control group) has been treated (*i.p*) with 0.9% NaCl solution. Prior the experiment, the animals were deprived of food but not water. Behavior of all animals was meticulously monitored during the first 4 h and daily during 14 days. Mortality and the lethal dose 50 (LD_{50}) were noted. At the end of the experiment, the animals are weighed and sacrificed. The blood is collected by the retro-orbital sinus for the determination of biochemical and hematological parameters. Organs such as liver, kidneys and heart are also isolated and weighed.

2.6. Statistical analysis

Results are presented as mean ± standard error of mean of six experiments (mean ±. SEM). GraphPad Prism 7 software, (Microsoft, San Diego California, USA) is used for statistical analysis of data and graphical representations. The significance differences between treatments is determine using to the variance analysis (ANOVA) of the Tukey-Kramer multiple comparison test. Difference is considered as statistically significant when $p < 0.05$.

3. Results

3.1. Output extraction of the EABV

The results showed that the output extraction of the aqueous EABV leaves is low (7.27 ± 0.24%).

3.2. Phytochemical screening of EABV

The different chemical compounds present in the EABV are presented in Table 1. The metabolites such us polyphenols, flavonoids, alcaloids, catechic tannins and saponosides are detected. But the extract does not contain sterols, triterpenoids and gallic tannins.

Table 1: Phytochemical screening of *Bambusa vulgaris* leaves

Secondary metabolites		Reactant/Tests	Results
Polyphenols		$FeCl_3$	+
Flavonoids		Cyanidin	+
Alcaloids		Dragendorff; Bouchardat	–
Tanins	catechic	Stasny	+
	Gallic	Acetate of sodium and ferric chloride	+
Saponosids		Physical	+
Sterols and Triterpenoids		Lieberman	–

Note: (+): Present; and (–): Absent.

3.3. Acute toxicity studies of EABV

3.3.1. Behaviours of rats

This study revealed several clinical signs of toxicity in rats treated at 300 mg / kg which increased at 2,000 mg / kg. Several symptomatic disorders like anorexia, acceleration of the heart rate, difficulty of locomotion with torsion, isolation and somnolence were observed.

3.3.2. Mortality and determination of LD_{50}

No mortality was recorded in rats treated at 300 mg / kg (Table 2). In contrast, all animals treated at 2,000 mg / kg dead before the end of the first day of the experiment. According to the harmonized global classification system of the OECD n °423, the LD_{50} of EABV is between 500 mg / kg and 2,000 mg / kg.

Table 2: Evaluation of acute toxicity of *Bambusa vulgaris*

Group	Treatments	Number of rats treated	Number of rats dead	Mortality (%)
1	NaCl 0.9 %	3	0	0
2	EABV (300 mg / kg)	3	0	0
3	EABV (2,000 mg / kg)	3	3	100

Note: Animals were divided into three groups of three rats. Group 1 (control) received NaCl 0.9 % while groups 2 and 3 received EABV at the doses of 300 mg / kg and 2,000 mg / kg. EABV: Aqueous extract of *Bambusa vulgaris* leaves.

3.3.3. Body weight and relative weight of organs

EABV increases gradually the body weight of treated group during the experimentation (Figure 1). However, it was lower and not significant (7.72 ± 1.31%) compared to the control (9.36 ± 1.4%) ($p > 0.05$).

Similarly, no significant difference ($p > 0.05$) is observed for the relative weight of the heart, liver and kidneys (Table 3).

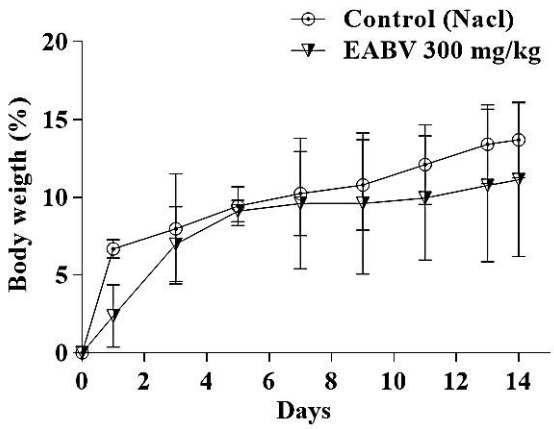

Figure 1: Effects of the aqueous extract of *Bambusa vulgaris* leaf on body weight in female Wistar rats

Note: Values are expressed as mean ± SEM, $n = 3$. ($p > 0.05$: no difference compared to the control group).

Table 3: Relative organs weight of rats after experimentation

Parameters	Control (NaCl 0.9%)	EABV 300 mg/kg
Heart	0.39 ± 0.03	0.40 ± 0.01
Kidneys	0.54 ± 0.009	0.52 ± 0.01
Liver	3.52 ± 0.24	3.17 ± 0.17

Note: Values are expressed as mean ± SEM, $n = 3$. $p > 0.05$: no difference compared to the control group; EABV: Aqueous extract of *Bambusa vulgaris* leaves.

3.3.4. Hematological parameters

EABV administered at 300 mg/kg increases significantly the mean corpuscular volume ($p < 0.05$) and the mean corpuscular hemoglobin content ($p < 0.01$). While, hemoglobin ($p < 0.05$) lymphocytes, erythrocytes, hematocrit ($p < 0.01$), leucocytes ($p < 0.001$) and platelets ($p < 0.0001$) levels decrease (Table 4).

Table 4: Effects of *Bambusa vulgaris* leaves on hematological parameters in rats

Hematological parameters	Control (NaCl 0.9%)	EABV 300 mg/kg
Erythrocyte (10^6/mm^3)	7.84 ± 0.24	5.20 ± 0.46**
Leucocyte (10^3/mm^3)	17.73 ± 0.77	9.25 ± 0.56***
Thrombocyte (10^3/mm^3)	1087 ± 5.86	260.5 ± 6.06****
Hemoglobin (g/100ml)	14.57 ± 0.72	11.77 ± 0.58*
Hematocrit (%)	46.20 ± 1.72	35.13 ± 1.1**
Mean corpuscular volume (fl)	59.07 ± 2.2	70.97 ± 1.37*
Lymphocytes (%)	78.67 ± 3.48	41 ± 4.36**

Table 4 (Cont.)

Hematological parameters	Control (NaCl 0.9%)	EABV 300 mg/kg
MCHC (g/100 ml)	31.57 ± 0.88	31.37 ± 0.09
TCMH (pg)	18.6 ± 1.14	27.33 ± 1.02**

Note: Values are expressed as mean ± SEM, n = 3. ( ) $p > 0.05$: not significant; (*) $p < 0.05$, (**) $p < 0.01$; (***) $p < 0.001$: Significant compared to control group. MCHC: Mean corpuscular concentration of hemoglobin; TCMH: Mean corpuscular hemoglobin content; EABV: Aqueous extract of *Bambusa vulgaris* leaves.

3.3.5. Biochemical parameters

Biochemical parameters are shown in Table 5. EABV, administred at 300 mg/kg has no significant effects on all parameters excepted ASAT ($p < 0.001$) and ALAT ($p < 0.05$) level which increases. Also, the biochemical analysis revealed a significant decrease of creatinine level in rat treated.

Table 5: Effects of *Bambusa vulgaris* leaves on biochemical parameters in rats

Parameters	Control (NaCl 0.9%)	EABV 300 mg/kg
ASAT (UI/l)	148.5 ± 4.91	206.7 ± 4.13***
ALAT (UI/l)	46.33 ± 0.88	50 ± 0.58*
Urea (mg/l)	0.91 ± 0.01	0.84 ± 0.04
Creatinine (mg/l)	6.67 ± 0.33	3.17 ± 0.37**
Na (mg/l)	139.5 ± 0.29	141 ± 0.58
K (mg/l)	4.43 ± 0.28	5.46 ± 0.3
Cl (mg/l)	109.3 ± 2.03	101.5 ± 2.29

Note: Values are expressed as mean ± SEM, n = 3. ( ) $p > 0.05$: no significant; (*) $p < 0.05$, (**) $p < 0.01$; (***) $p < 0.001$: Significant compared to control group. Cl: chlorine; Na: sodium; K: potassium; ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase; EABV: Aqueous extract of *Bambusa vulgaris* leaves.

4. Discussion

Natural substances extraction is of great interest because this method makes it possible to obtain a concentrate of active ingredients responsible for the many virtues of the plant (Bonnaillie *et al*., 2012). Decoction has been chosen as a method of extraction because several ethnobotanical surveys have revealed that this technique is recommended in the treatment of several diseases, including arterial hypertension and malaria (Kimpouni *et al*., 2018; and N'Guessan *et al*., 2009). It is therefore obvious that this kind of extraction does not make it possible to extract in large numbers the various secondary metabolites of this plant. Therefore, the secondary metabolites contained in the leaves of *Bambusa vulgaris* would have more affinity for other solvents such as methanol. Kumar and Suseela (2011) obtained a 15% output with the methanolic EABV leaves. Besides solvents, the contact time of the powder with water could be the cause of this low output (Abo, 2013).

The study of medicinal plant properties is associated with a phytochemical screening. It's carried out by several methods including the tube characterization tests. This method is based on the appearance of coloring, precipitation and foam formation. The results revealed the presence of different secondary metabolites such as polyphenols, flavonoids, catechic tannins, alkaloids and saponosides in the decocted leaves of *Bambusa vulgaris*. On the other hand, the aqueous extract is devoid of sterols, triterpenoids and gallic tannins. The studies of Tripathi *et al*. (2015) and Kumar and Suseela (2011) revealed that *Bambusa vulgaris* leaves contain sterols and terpenoids. Conversely, this plant not contains polyphenols and tannins. The presence or absence of these metabolites may be due to the edaphic conditions of the collection site of *Bambusa vulgaris* leaves, the period of harvest and the type of extraction used.

The acute toxicity study of EABV reveals that this extract injected at 300 mg/kg and 2,000 mg/kg causes several behavioral disorders in rats. However, this plant does not modify the behavior of animals when

administered orally (Kumar and Suseela, 2011). It is therefore obvious that the route of administration is a very determining factor in the onset of the toxic effects of this plant. Administered orally, the extract involvement of digestive enzymes and the different metabolic processes of chemicals in the plant. However, EABV directly joins the bloodstream and organs by intraperitoneally route. The consequences are immediate. Also, the presence of bioactive substances like alcaloids could justify the appearance of these clinical signs (Baulac *et al*., 2001; Luis Blanc *et al*., 1988 and Yinyang *et al*., 2014). This clinical signs are also observed by Zougrou (2017) when he studied the toxicological effect of the aqueous extract of *Cinestis ferruginea* (Connaraceae) in mice.

No mortality was recorded at 300 mg / kg. In contrast, all animals treated at 2,000 mg / kg dead before the end of the first day of the experiment. According to the harmonized global classification system of the OECD (2001), EABV is classified in category 4 with a LD_{50} between 500 mg / kg and 2,000 mg / kg. As a result, *Bambusa vulgaris* is judged to be of low toxicity when administered intraperitoneally (Diezi, 1992). This plant is therefore less toxic than *Swartzia madagascariensis* (Ceasalpiniacea), *Datura stramonium* (Solanaceae) and *Terminalia ivorensis* (Combretaceae). The LD_{50} are 5.99 mg / kg (Traoré *et al*., 2002), 203 mg / kg (Allouni, 2001) and 200 mg / kg (Zaza *et al*., 2018) respectively.

Besides these parameters, the variation of the body mass is an indicator of the adverse effects of this plant (Vahalia *et al*., 2011). These results indicate that *Bambusa vulgaris* has no toxicity to body weight of rats.

The identification of any morphological abnormality of an organ is essential in toxicology in so far as it reveals the toxicity of a substance. This study focused on the evaluation of the relative weight of the heart, liver and kidneys as these organs contribute to the proper functioning of the body. In addition, vascularized organs are the targets of the adverse effects of drugs (Lapointe, 2004). No atrophy or hypertrophy was observed at the end of the experiment.

The evaluation of hematotoxicity in the preclinical tests is very essential because it reveals higher risks in humans (Olson *et al*., 2000). Hematological analyzes revealed that EABV at 300 mg / kg affects all parameters except the mean corpuscular concentration of hemoglobin. This dysfunction could affect hematopoietic system.

For biochemical data analysis, the extract increases on the one hand the serum ASAT and ALAT level and decreases on the other hand the creatinine level. The increase of this enzyme in the serum of the treated indicates a hepatic, cardiac or renal damage (Hilaly *et al*., 2004; Ramaiah, 2011; and Yakubu *et al*., 2005).

5. Conclusion

Ultimately, the decoction, used as extraction technique allows to obtain a low output. It has been revealed that *Bambusa vulgaris* leaves contain several molecules responsible for its pharmacological effects. The LD_{50} obtained is between 500 mg / kg and 2,000 mg / kg. This extract is therefore weakly toxic when injected intraperitoneally. The hematological data showed that the acute administration of *Bambusa vulgaris* (*i.p*) at 300 mg / kg affects significantly the hematopoietic system. The ALAT and ASAT levels increased markedly, although biometrics of the kidneys and liver showed no change. Therefore, the exploration of the pharmacological effects of this plant requires the use of much lower doses.

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