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Acute toxicity and indirect effects on the NA^+/K^+ ATPase pump of chloroform extract of *Mansonia altissima* (Sterculiaceae) in rabbits

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Abstract
Mansonia altissima bark is known as poison but also for these multiple effects including cardio- protective effect. This study determine to acute toxicity and effect on the Na^+/K^+ of chloroform extract of *Mansonia altissima* bark. Acute toxicity of chloroform extract of *Mansonia altissima* was conducted. Then, ion pool was performed by administrating increasing doses (4.7-75 mg/kg) of chloroform extract in 36 rabbits devised in 7 lots and ion (Ca^{2+}, Mg^{2+}, Na^+ and K^+) were determinate by spectrophotometer analyzer. The results showed a lethal lose 100 (DL_{100}) of 125 mg/Kg, lethal lose 50 (LD_{50}) of 92.5 mg/Kg and maximum tolerated dose (MTD) of 75 mg/Kg in mice type albino. The value of LD_{50} indicates that chloroform extract has moderate toxicity. Results of ion pool showed significant ($p<0.05$) decrease of Na^+ and non-significant Ca^{2+} concentrations in plasma, associated with non-significant first increase ($p<0.01$) in plasma of K^+ concentration for lower doses of extract at 18.7 mg/kg of body mass weight. Plasma concentrations of Mg^{2+} were also experienced a non-significant increase ($p <0.10$) in relation to the pump Na^+/K^+ ATPase inactivation. These results relate that administration of non-toxic doses of chloroform extract of *Mansonia altissima* bark inhibit action on Na^+/K^+ ATPase and myocardial contraction.

Keywords: *mansonia altissima*, acute toxicity, electrolyte metabolism, cardiovascular disease

1. Introduction
The use of natural substances by traditional healers often falls mystical practices handed transmitted from generation to generation. The preparation of these drugs from these substances remains, even today, in compromising secret as well as the sustainability of these ancestral knowledges [1]. So that in Africa, the gradual disappearance of healers, is a danger to the preservation of knowledge and therapeutic practices [2]. In our country, where the oral tradition is the only way of expression for the majority of these healers, the study of traditional medicines is essential and urgent. It's the case *Mansonia altissima* whose extracts of bark are known as poisons which are used for hunting purposes by certain western populations of Côte d'Ivoire [3, 4, 5]. This plant has also of pharmacological doses and therapeutic value on major cardiovascular diseases, because of its cardiotonic properties on peripheral blood vessels [6, 7].
So, the dust produced when processing the carpentry wood may cause dermatitis, nose bleed, throat irritation, asthma and eczema, reactions strong increasingly on repeated exposure and disorders heart [4]. Moreover, studies on the ethanol extract of the wood showed hepatotoxic and blood-toxic effects when it administered orally to rats [8]. Ethanol and chloroform extracts of bark howed very high toxicity of many mammals [8, 9]. This is also the case for dermatological and infectious diseases where extracts of bark also inhibit growth of *Mycobacterium tuberculosis*. Seen its antibacterial properties, the addition of the bark in small amounts in chicken feed to replace antibiotics trade improved the feed conversion ratio [10].
These information's have guided this study whose purpose is to determine the acute toxicity parameters of chloroform extract of *Mansonia altissima* bark and to study indirect effects of this extract on the Na^+/K^+ ATPase pump respectively in Swiss mice and New Zealand rabbits.

2. Material and Methods
2.1 Plant material
The barks of *Mansonia altissima* of were collected in forest areas of Daloa (Côte d'Ivoire), and authenticated by comparison with herbarium specimens already existing in National Floristic Center (C N F) of Félix Houphouët-Boigny University (Côte d'Ivoire) [6, 7]. These barks were washed with distilled water and were shade-dried at room temperature during ten days (6 weeks), and subsequently reduced to coarse powder using a grinder and stored at room temperature [7, 11].

2.2 Animal material
To assess the acute toxicity of chloroform extract of *Mansonia altissima*, the experimental protocol animals used was in accordance with the guidelines for ethical care of experimental animals of the OECD [12]. 70 mice Swiss type (male and female) about 8 weeks old with an average weight of 20 ±0.7g. To study effects of *M. altissima* onion and electrolyte,

were used 36 New Zealand rabbits Cunistar type of 1.5 ±0.24 Kg, aged of 8 weeks from farming Bingerville (Côte d'Ivoire). All animals acclimatized for two weeks during which they receive an average of 160 g daily of a suitable supply of IVOGRAIN origin.

2.3 Preparation of chloroform extract

One hundred grams (100 g) of powder were macerated in 1000 mL of distilled water during 24 hours. The homogenate were filtered three times in succession absorbent cotton and then once on Wattman paper. Then, the filtrate was lyophilized using freeze-dryer model Telstar-LyoQuest-55 to obtain the dried aqueous extract [7, 11]. From this crude extract, the chloroform extracts of *Mansonia altissima*, was prepared according to own technique laboratory and chloroform portion isolated by fractionation [7, 11].

2.4 Purification of the chloroform extract

In the context of a purification process, it has been realized on the chloroform extract of *Mansonia altissima* by thin layer chromatography in several steps of which the last one on a silica gel column with an eluent made up of chloroform and 15% of methanol. The product is drying object during 1 h 30 mn in an oven (115° C). We obtained by revelation with the antimony trichloride ($SbCl_3$) that the spot lights are ranged from yellow-green to brown-yellow, which tended to disappear [7, 11].

2.5 Phytochemical screening

Phytochemical sreening was carried out on the chloroform extract to characterize the different chemical groups present in a plant extract with pharmacological interest [11]. These are chemical reactions that help identify the presence of chemical substances. These tests have focused on research the following main chemical groups reported in Table 1.

Table 1: Products of characterization of main chemical constituents of chloroform extract of *Mansonia altissim*

Secondary metabolites	Reaction caracterizing
Total phenols	Reaction of Folin-Ciocalteu
Flavonoids	Reaction at cyanidin
Alkaloids	Reaction of Dragendorff and Bouchardat
Tanins	Reaction of Stiasny
Saponins	Test de production de mousse
Quinones	Reaction of Borntraeger
Sterols and triterpenes	Reaction of Liebermann
Cardiotonic Glycosides	Reaction at liqueur de Fehling

2.6 Acute toxicity study

2.6.1 Experimental animal

The previously separate mice were placed in plastic cages containing wood shavings renewed every 3 days. The 70 mice (male and female) were acclimatized for during 4 weeks and divided into 7 homogeneous groups of 10. They were fasted for 24 hours before administration of the extract [9, 12].

2.6.2 Animal treatment

The administered solutions were prepared the day before. Mice in the control group (group 1) received 0.1 mL of chloroform solution. The others lots of mice have received different concentrations of chloroform extract of *Mansonia altissima* based on weights of different lots. These solutions were administered intraperitoneally using sterilized insulin-syringes. The animals thus treated are subjected to continuous monitoring during the 2 hours to raise the number of deaths and clinical signs for each lot [9].

2.6.3 Assessment of acute toxicity

Concentrations of chloroform extract of bark of *Mansonia altissima* were prepared based on the principle that concentrations administered should be reduced to the body weight of the mice saw that the injected doses in mg/kg of body weight (b.w.). Thus, a mother solution of chloroform extract of *Mansonia altissima* was obtained from the dissolution of 1 gram of product in 100 mL of chloroform, corresponding to 1% or a concentration of 10 mg/mL. From this stock solution, different dilutions were performed to obtain concentrations corresponding to doses of 75 - 87.5 – 100 - 112.5 – 125 and 137.5 mg/kg of body weight [9].

2.6.4 Determination of lethal doses

The 70 mice of 20 ±0.7g divided in 7 lots of 10 mice were subjected to intraperitoneal injections. Toxicity tests were conducted in mice by injecting chloroform extract dilutions of 1% for the determination of lethal doses (LD) and tolerable doses (TD). The 7 lots were treated with dosages of chloroform extract of *Mansonia altissima* by using respective concentrations of 0.15 to 0.55 mg/mL. Acute toxicity parameters determined in this study were maximum tolerated dose (MTD), lethal dose 50% effect (LD_{50}) and lethal dose to 100% effect (LD_{100}). They were obtained from the curve trevan given by the percentage of mortality of mice as a function of logarithm of doses administered [9, 13].

2.7 Ion pool or electrolyte mechanism

2.7.1 Experimental animal

The rabbits (36) were divided equally into 6 groups of 6 rabbits, including a control group. They have been conditioned in accordance with international standards of toxicological expertise drugs. The doses administered intraperitoneally were less than or equal to DMT of chloroform extract obtained after the completion of acute toxicity assess. This study involved the use of chloroform extract of *Mansonia altissima* at 75.0 mg/kg of body weight, similar dose those found by Guédé-Guina [6]. Thus, concentrations of chloroform extract of *Mansonia altissima* administered to different groups of rabbits are 4.69 - 9.37 - 18.75 - 37.5 and 75 mg/kg of body weight.

2.7.2 Terms sampling

The samples are taken on an empty stomach in the marginal vein, once a week with sterile 23 G needle. The collected blood was centrifuged at 3000 revs/min for 10 min. the obtained were aliquoted to be stored at -20 °C freezer.

2.7.3 Ionic parameters assays

The ion parameters analyzed were calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}). Sodium and potassium were analyzed using spectrophotometer SEAC fp 20 to temperature of 2000 °K with reagents "Corning® " and read at 589 λ (nm) for sodium and 767 λ (nm) for potassium. Reactive "bioMérieux® " were used in a UV visible Hitachi 704 automatic analyzer for determination of calcium and

magnesium through colorimetric methods in alkaline medium. The dosage of calcium is made in presence of O-cresol phthalein reading with 500 λ (nm) and calmagite followed a reading at 530 λ (nm) for magnesium.

Statistic analysis

In the study the values presented are expressed by assigned average standard deviation of the average. A comparison of average statistical analysis is made according to the Student's t test. The difference is seen when significant at $p \leq 0.05$.

3. Results

3.1 Phytochemical analysis of chloroform extract of *Mansonia altissima*

It is observed that the chloroform extract purified bark of *Mansonia altissima* contains alkaloids, tannins, saponins, quinones, terpenes, polyphenols, flavonoids and cardiac glycosides, which are secondary metabolites (Table 2).

Table 2: Secondary metabolites of chloroform extract of M. altissima

secondary metabolites	Chloroform extract
Alkaloids	+
Tanins	+
Saponins	+
Sterols	+
Polyphenols	+
Quinones	+
Terpenes	+
Flavonoids	+
Cardiac glycosides	+

(+) Presence, (-) Absence

3.2 Acute toxicity

3.2.1 Mortality depending on dose

It is observed a growing rate of mortality in experimental animals that is proportional to the increase in the concentration of the chloroform extract of *Mansonia altissima* administered with a maximum at 137.5 mg/kg of mouse body weight (Table 3).

Table 3: Mouse mortality as a function of the dose of the chloroform extract of *Mansonia altissima*

Groups of animals	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Number of animals used	10	10	10	10	10	10	10
Injected dose of the product (mg/kg)	0	75	87.5	100	112.5	125	137.5
Administered concentration (mg/ml)	0.1	0.15	0.17	0.20	0.22	0.25	0.55
Amount of injected (mg/mouse)	0	1.5	1.75	2	2.25	2.5	2.75
Number of recorded deaths	0	0	3	6	8	100	10
Percent mortality	0	0	30	60	81	100	100

3.2.2 Determination of LD_{50}

The projection on axis curve allowed deducting acute settings of acute toxicity in mice treated with chloroform extract of *M. altissima*. We obtained one DL_{50} of 92.5 mg/kg of body weight which was very significant (Figure 1).

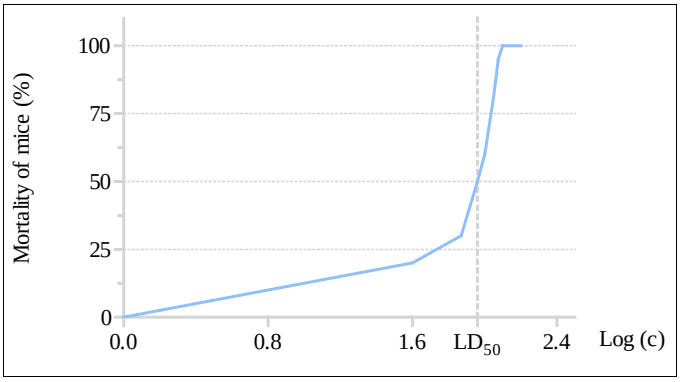

Fig 1: Curve mortality trends mice based doses of chloroform extract of *Mansonia altissima* bark c: injected concentrations (mg/kg/b.w.); LD50: lethal dose 50%.

3.3 Effects on plasma ion pool rabbit (K^+, Na^+, Ca^{2+})

The study of electrolytes shows the increase K^+ ion in plasma when the injected concentration product increase. Conversely, the concentrations of Na^+ and Ca^{2+} decrease with increasing concentrations of injected chloroform extract (Figure 2). The plasma concentration of Mg^{2+} ion did not change during the study.

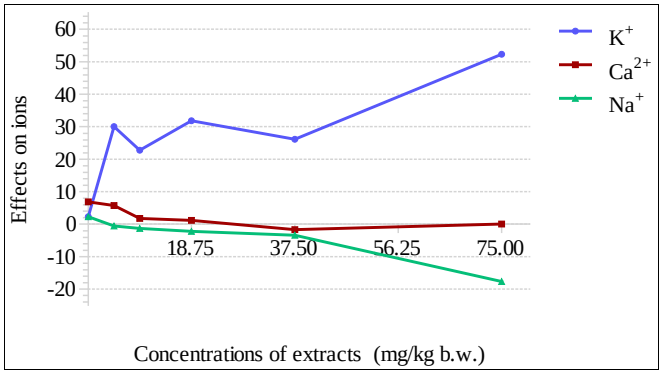

Fig 2: Variations of plasma electrolytes studied according to doses injected to rabbits

4. Discussion

Substances obtained after chloroform extraction and purification may be used for producing chemical characterization studies, toxicological and physiological on different animal models of organs such as the rabbit in order to understand their action mechanisms. Phytochemical analysis was resumed with a view to confirming preliminary results [11]. Sorting phytochemical highlighted chemical groups whose alkaloids and cardiac glycosides that have cardiotonic and vasodilatory properties or on peripheral blood vessels. Among the above-mentioned alkaloids has been identified. Also, extract of *Mansonia altissima* bark contains a cardiac glycoside, the mansonine which is a toxic compound that is similar to cardenolides [7]. Digitalis and ouabain from

Strophanthus. Its aglycone has been identified, it is strophanthidin.

Acute toxicity of chloroform extract of *Mansonia altissima* on mice

The analysis of results indicates a growth of mortality rates progressively increase according to dose-response of chloroform extract 1% of *Mansonia altissima* bark. Indeed, the mortality rate increased 30% dose of 87,5 mg/kg to 100 mg/kg, and 19% of the dose 112.5 mg/kg to 125mg/kg. This allows deducing a dose-response effect of chloroform *Mansonia altissima* extract on mice [14]. The different toxicity tests of the chloroform extract of bark of *Mansonia altissima* allowed us to obtain the following results with the maximum tolerated dose is 75 mg/kg body weight; the lethal dose 50 of 92,5mg/kg and the lethal dose of 100, 125 mg/kg. Acute toxicity parameters thus obtained show that at doses between 0 and 75 mg/kg body weight, there is a lack of mortality in mice. However, at doses greater than 75mg/kg of body weight, he was found dead mice after a definite time.

TMD obtained appears as one tolerated by the body and could therefore potentially be used for experimental basis in a biological safety study on other animal models. These results obtained with the DMT of 75 mg/kg body weight corresponding to a concentration of 1.5 mg /20 g. They are approximate to the minimum concentration of chloroform extract of *M. altissima* (DMT = 1.6 mg/25g equivalent to 64 mg/kg) reported by Guédé-Guina [6, 15]. Moreover, Guédé found after chloroform extract of Directors of *Mansonia altissima* bark 1%, an LD_{50} of 77 mg/kg of Pc (or 1,93mg/25g) and DL_{100} 89 mg/kg of body weight (or 2.23 mg/ 25 g) in mice.

Thus, the value of 92,5mg/kg body weight obtained for the LD_{50} in mice is used to classify the chloroform extract of *Mansonia altissima* bark of moderately toxic substance according to the scale of Gosselin toxicity classes, and Smith Hodge [12, 16]. Moreover, for this LD_{50} value, a person of 70 kg should receive 92.5 mg/kg x 70, or 6475 mg of product as a single dose to run the same risks. This dose of 6, 475 g of chloroform extract of *Mansonia altissima* bark on the classification scale Gosselin *et al*. [17], could be classified almost not toxic to humans and can be then used for physiological test.

These results show that the chloroform extract of *Mansonia altissima* bark is inactive in vivo at doses below 75 mg/kg body weight and allow to see that the chloroform extract of *Mansonia altissima* single doses exert its toxic action over a period ranging from 35 minutes to 2 hours after injection according to data observed clinical signs. Beyond 2 hours, treated mice surviving each batch are within a recovery phase which becomes complete after 48 hours. Given this observation, it appears that the animal organism could detoxify after 48 hours either by making the substance harmless, either by completely eliminating the natural excretion routes.

Before administration of chloroform extract of *Mansonia altissima*, ionic plasma parameters in rabbits about sodium and potassium were respectively between 136.95 mg/l and 143.45 mg/L and 3.67 mg/l 4.57 mg/L against 137.93-145.85 mg/L and 3.51-4,27mg/L. Serum calcium in our study ranged from 89.75 to 98.25 mg/L against 89.57- 98.43 mg/L. These values at rest join those obtained of the New Zealand rabbit–Cunistar in Côte d'Ivoire by Coulibaly [3]. Thus, sodium, potassium, calcium and magnesium in serum are respectably 140.2 ±3,25 mEq/L; 4.12 ±0.45 mEq/L; 94 ±4.25 mg/L and 15.72 ±3.74 mg/L. These values observed in rabbits of tropical areas do not appear with Boucher [5] data indicative concerning plasma magnesium values between 57 mg/L and 47 mg/L and potassium those between 6 mEq/L and 5.5 mEq /L in rabbits.

The administration of chloroform extract of *Mansonia altissima* in rabbits showed a gradual decrease in sodium administered at the lowest concentration (4.7 mg/kg) (passer) 144.7 mg/L to 116 mg/L average to 75 mg/kg. This decrease was significant ($p < 0.05$). Plasma concentrations of potassium on the other hand there is a progressive increase becomes significant ($p < 0.01$) in the administration of a product concentration of 18.7 mg/kg body weight body. Calcium and magnesium respectively and *undergoes* a non-significant decrease marked increase ($p < 0.10$) in the administration of increasing doses of chloroform extract of *Mansonia altissima*.

Plasma variations of different electrolytes (Na^+, K^+, Ca^{2+} and Mg^{2+}) on administration of chloroform extract of *Mansonia altissima* is explained the mechanism action of these ions on the cardiac tissue by the inhibiting action on the Na^+/K^+ ATPase pump contained in the chloroform extract [18]. The non-significant increase constated plasma concentration of magnesium after injection of increasing doses of chloroform extract is also related to the inhibition of the Na^+/K^+ ATPase pump membrane whose activation involves the Mg^{2+}.

The cardotonic activity of the chloroform extract of *Mansonia altissima* is due to the presence of cardiac glycosides digitalic or revealed by phytochemical screening. The digitalic, a therapeutic class of drug used in cardiology is a related substance digitalin, derived from digital (plant). His cardiac mechanism of action is manifested by a positive inotropic effect (increasing constructability) by inhibiting the Na^+/K^+-ATPase infarction, cardiac tissue driver, vascular smooth fibers and certain other tissues such as red blood cell.

Indeed, Mansonine structural analogue of the active compound ouabaïne [18, 19] is a cardiac glycoside that binds competitively to the K^+ binding site there by blocking the ATPase activity. Na^+ can no longer leave the cell accumulates in the cytoplasm in the membrane, leading to a decrease in the concentration gradient between Na^+ extra and intracellular for Na+ intracellular due to the continuation of the passive diffusion of Na^+ which explain the decline observed plasma concentration. It is observed a slower exchange Na^+/Ca^{2+} with less output of Ca^{2+} or a reversal of the exchange Na^+/Ca^{2+} to allow entry of Ca^{2+} into the cell. The increase in the concentration of intracellular Ca^{2+} activates the contractile elements responsible for the positive inotropic effect [20].

Therefore, the amount of calcium stored in the sarcoplasmic reticulum and available for contractile elements during the given cell depolarization cycle is increased. The myocardial contractility is thereby improved [4, 6]. It is therefore found that the plasma concentrations of electrolytes studied are closely connected with the movements across the cell membrane that are mediated by the Na^+/K^+ ATPase pumps and the calcium pump [20].

Ultimately, the secondary metabolites of chloroform extract of *Mansonia altissima* meets the mansonine end of the purified extract of Mansonia altissima by Guede-Guina in 1992 and showed an ionic pool with inhibition of the Na^+/K^+ ATPase membrane in rabbits. This effect appears that this drug is a

potentially offering of the new drug therapy [20, 21, 22] for cardiovascular diseases as suggested by some studies [22].

5. Conclusion

The chloroform extract *of Mansonia altissima* bark is a moderately toxic substance according to Hodge and Sterner classification scale. The moderate nature of its toxicity would give this phytomedicine the possibility of therapeutic use should be safe at doses lower than 75 mg/kg body weight. The chloroform extract of *Mansonia altissima* bark owe this cardiotonic activity it content of glycosides cardiotonic. Further studies should be directed towards a better understanding and mastery of its physiological properties to use for medicinal purposes in cardiovascular diseases.

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7. References

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