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Research Article

Acute Toxicity in Mice and Effects of a Butanol Extract from the Leaves of *Blighia Unijugata* Bak. (Sapindaceae) on Electrocardiogram of Rabbits

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Abstract: *Blighia unijugata* is a plant used in traditional medicine to treat many pains and diseases such as fever, headaches, kidney pain and stiffness, dizziness and high blood pressure. No or few studies were carried out on its effects on cardiovascular system. So, the present work was aimed to evaluate the effects of a butanol fraction from the leaves of this plant on the electrocardiogram of rabbits and to assess the acute toxicity in mice. The registration of electrocardiogram was implemented by an electrocardiograph (CARDIOFAX ECG-6851K Nihon Kohden, Japan) and the acute toxicity was determined by the methods of Miller and Tainter and Dragsted and Lang. The results showed that the butanol fraction from the leaves of *Blighia unijugata* (BFBu) was not toxic by oral and intraperitoneal administrations. The LD50 determined by oral tract was 20.14 ± 0.44 g/kg b.w. by the graphic method and 21 ± 0.54 g/kg b.w. by the calculation method. The intraperitoneal administration of the extract permitted to determine the LD_{50} of 5.628 ± 0.29 g/kg b.w. by the graphic method and 5.044 ± 0.28 g/kg b.w. by the calculation method. On rabbit electrocardiogram, BFBu elicited decreases of P, QRS, T waves and cardiac frequency. It caused increase of QT interval and. PQ interval was not affected. The effects induced by BFBu were partially inhibited by atropine suggesting the presence of cholinomimetic substances in the extract. The phytochemical screening revealed the presence of sterols and polyterpenes, polyphenols, flavonoids and saponins. These compounds particularly polyphenols and flavonoids could be responsible for the inhibitory effects on the electrocardiogram of rabbit justifying the use of this plant in traditional medicine on the cardiovascular system.
Keywords: *Blighia unijugata*, acute toxicity, ECG, phytochemical screening.

INTRODUCTION

In Africa, nearly 80% of populations use traditional medicine for their health needs [1-4]. Plants constitute the main source of the practice of this medicine. Despite the increased use of medicinal plants for healthcares, only a relatively small number of species has been studied for possible conventional medical applications. Data on safety and efficacy are available for a limited number of plants, thereby exposing people to all kinds of hazards [5]. *Blighia unijugata* is among the great source of plants used. It is a forest species widespread in tropical Africa, from Sierra Leone to Cameroon. It is found in Côte d'Ivoire in the secondary formations in reforestation [6-8]. *Blighia unijugata* is a shrub with 6 to 9 m tall, but sometimes up to 30 m tall, with a dense crown. The bark is gray or brown, generally smooth, fine, sometimes with warts. The leaves are reddish when young and become shiny green when adult [6, 9]. Many traditional indications of this plant have been reported. *Blighia unijugata* is used as a vegetable and also in the treatment of fever, nausea and vomiting, leprosy, eyes aches, coughing, headaches, rheumatism, kidney pain and stiffness, dizziness and high blood pressure [6, 8]. Ethanol extracts of roots, stem barks and leaves showed antibacterial activity, especially against *Staphylococcus aureus* [10]. The study of the toxicity of oils extracted from seeds and aril of *Blighia unijugata* on hematological and biochemical parameters and histopathological analysis in rats revealed that these oils induced no signs of clinical toxicity [11]. The presence of steroids, saponins and tannins was shown in all these extracts [11]. This study was aimed to assess the acute toxicity of a butanol extract of *Blighia unijugata* in mice, to evaluate its effects on the electrocardiogram of rabbits and to screen for phytochemical compounds present in the extract and probably responsible for its effects.

MATERIALS AND METHODS

Animals

Mice (*Mus musculus*) weighing between 20 and 30 g and rabbits (*Oryctolagus cuniculus*) weighing 2 ± 0.2 kg were used respectively for acute toxicity and electrocardiogram. They were bred in Animal house of Animal Physiology, Pharmacology and African

Pharmacopoeia of the University of Nangui Abrogoua (Former University of Abobo-Adjamé, Abidjan, Côte d'Ivoire) according to the principles for the care and use of laboratory animals of the Ethical Committee of the University (Nangui Abrogoua, Abidjan, Côte d'Ivoire).

Plant material

Fresh leaves of *Blighia unijugata* (Sapindaceae) were collected in Abidjan (Côte d'Ivoire) in June 2009. Taxonomical identification of those leaves was established by Professor Aké Assi Laurent from the National Floristic Centre of University of Felix Houphouet Boigny, Cocody- Abidjan, Côte d'Ivoire, voucher n°165 of Côte d'Ivoire national herbarium.

Plant extraction

Fresh leaves of *Blighia unijugata* were dried under shade and powdered with an electric grinder (Culatti, France). The extraction process was implemented according to the methods described by some authors [12-14]. One hundred grams (100g) of the leaves powder were macerated for 48 hours in 2l of ethanol 96% under magnetic shaking. The resulting solution was filtered (Whatman n°1) and concentrated under reduced pressure using a rotary evaporator (Büchi R110, type MKE 6540/2) at a temperature of 60°C. After drying in an oven at 45°C for 48 hours, 17.3 g of total ethanol extract were obtained. Ten grams (10 g) of total ethanol extract were dissolved in 200 ml of boiling distilled water. The mixture was homogenized under magnetic shaking for 15 minutes at 27 ± 2°C. The aqueous solution obtained was then exhausted for 10 minutes at 27 ± 2°C with 200 ml of hexane to give two phases after decantation: a residual aqueous phase and an organic phase. The residual aqueous phase was again treated for 10 minutes at 27 ± 2°C with 200 ml of chloroform to give two phases: an organic phase and a residual aqueous phase. The same operation was continued by successively treating the residual aqueous phase with ethyl acetate and then with butanol. Each of these organic phases and the final residual aqueous phase obtained were concentrated under reduced pressure at 60°C using a rotary evaporator as described above. Organic fractions obtained from these partitions were hexane fraction (0.6 g), chloroform fraction (1.1 g), ethyl acetate fraction (1.6 g), and butanol fraction (3.18 g) and the aqueous fraction was the final residual aqueous fraction (3.12 g). The butanol organic fraction (BFBu) was found to be the most interesting fraction because it was the most active on arterial blood pressure of rabbits during the preliminary tests and was retained for pharmacological and phytochemical tests.

Chemical

Atropine was purchased from Prolabo (France).

Data analysis

All values were expressed as mean ± standard error on the mean (m±sem). Statistical analysis and graphics were carried out using the software GraphPad Prism 5.01 (San Diego California, USA). The significance of the differences observed between the doses was achieved by analysis of variances (ANOVA) followed by Tukey test. The differences between the doses were considered statistically significant when $p<0.05$.

Acute toxicity study by oral and intraperitoneal routes

Mice were distributed into one control group and six treated groups containing ten animals (5 males and 5 females) per group. They were fasted for 18 hours prior to experiments. The control group received distilled water orally while each treated group received orally a single administration of BFBu at the following doses: 16, 18, 20, 22, 24 and 26 g/kg body weight (b.w.). Behavioural changes of the 6 treated groups were observed every 30 min for a period of 2 hours after administration of the extract and mortality rate were recorded for 24 hours post treatment [15]. The method of Miller and Tainter and that of Dragsted and Lang were used to determine the LD_{50} [1, 2].

The same protocol was used for intraperitoneal administration except that each mouse in the control group was treated with 0.5 ml isotonic solution of NaCl 9 ‰ intraperitoneally and the 6 other groups were treated with a single intraperitoneal administration of BFBu at 1, 2, 4.065, 6.024, 10 and 12.048 g/kg b.w.

Registration of the global electrical activity (ECG) of the rabbit

The methods were as previously described by some researchers [16-19]. The electrocardiogram of the rabbit was recorded by the technique of external electrodes used in the human practices and adapted to the rabbit [16]. Briefly, the saphenous vein of the anesthetized rabbit by an intraperitoneal injection of 40% ethyl urethane (1 g/kg body weight) was intubated in order to administer plant extract [16]. The armpits of the anterior limbs and the groin of the posterior limbs were shaved and cleaned with 90% ethanol. After applying electrolytic dough, four electrodes were put and bound to the four sockets of the registration cable connected to the electrocardiograph (CARDIOFAX ECG-6851K, Nihon Kohden, Japan). The studied parameters (P, QRS, T waves, PQ, QT intervals and cardiac frequency) were recorded from the DIII derivation of the standards or bipolar Einthoven derivations on thermo sensitive paper, at constant speed (25 mm/s). The butanol fraction of *Blighia unijugata* (BFBu) and atropine were dissolved in Mac Ewen solution of the following composition (mM): NaCl 130; KCl 2.5; $CaCl_2$ 2.4; NaH_2PO_4 1.18; CO_3NaH 11.9; $MgCl_2$ 0.24; $C_6H_{12}O_6$ 2.2 with a pH adjusted to 7.4. The tested doses of BFBu were 0.5, 2.5, 5, 10, 20, 30, and 40 mg/kg b.w. Atropine was used at $5x10^{-8}$, $5x10^{-6}$, $5x10^{-4}$ and $5x10^{-2}$ mg/kg b.w.

Phytochemical screening

The butanol fraction of *Blighia unijugata* (BFBu) was screened for the presence of polyphenols, tannins, flavonoids, saponins, alkaloids, sterols and ploy terpenes, reduced sugar, coumarines, quinones and cardiotonic glycosides. Detection of these constituents was achieved as described by Longanga *et al*. and Bekro *et al*. [20, 21].

RESULTS

Acute toxicity of BFBu by oral tract

No toxicity symptoms were noticed in mice after the oral administration of BFBu at the dose of 16 g/kg b.w. No mortality was registered at the same dose after 24 hours. However, the administration of BFBu at doses ranging from 18 to 26 g/kg b.w. induced some behavioural changes and caused dose dependent mortality in mice. Indeed, at 18 and 20 g/kg b.w., animals had difficulties in moving, tended to gather in a corner of the experimental cages, fed and drank very little. These signs were observed 1hour post treatment. At 22, 24 and 26 g/kg b.w., animals were immobile and refused to feed 30 min after administration of the extract. The first deaths occurred 2 h 30 min post treatment at the dose of 18 g/kg b.w. and 3 deaths were recorded. Death occurred after convulsions and jerky breathing and animals remained lying down on their back. At the dose of 20 g/kg b.w., 4 mice died 4 hours after oral administration of BFBu. At dose of 26 g/kg b.w., no animals survived. The mortality rate of mice was then 100%. This dose corresponded to the lethal dose that caused mortality of all mice in the designated group. The death rate of one experiment is shown in table 1. This experiment was repeated 3 times and the LD_{50} determined graphically by the method of Miller and Tainter was 20.14 ± 0.44 g/kg b.w. The calculation method of Dragsted and Lang gave a value of 21 ± 0.54 g/kg b.w. as LD_{50}. There is no significant difference between the two values of LD_{50} ($p>0.05$).

Table 1: Acute toxicity of BFBu by oral tract in mice

Groups of 10 mice	Dose of BFBu (g/Kg b.w.)	Number of mice dead	Mortality %
1	DW (Control)	0	0
2	16	0	0
3	18	3	30
4	20	4	40
5	22	6	60
6	24	9	90
7	26	10	100

Acute toxicity of BFBu by intraperitoneal administration

The intraperitoneal injection of BFBu at the dose of 1 g/kg b.w. did not elicit any changes in the behaviour of mice. The intraperitoneal administration of BFBu for doses ranging from 2 to 12.048 g/kg b.w. triggered diminution of mobility and piloerection as compared to control group. These effects were noticed 30 min after injection of the extract. Two hours after injection of BFBu from 6.024 to 12.048 g/kg b.w., mice became completely immobile, gathered in a corner of their cages and did not feed and drink any more. First deaths were registered at 2 g/kg b.w. six hours post treatment. Death occurred after convulsions and jerky breathing and animals remained lying down on their side. Death rate increased dose dependently from 2 to 12.048 g/kg b.w. The lethal dose 100% (DL_{100}) was reached in the group that received the dose of 12.048 g/kg b.w. The death rate of mice for one experiment is shown in table 2. This experiment was repeated 3 times. The LD_{50} determined by the graphic method of Miller and Tainter was found to be 5.628 ± 0.29 g/kg b.w. while the calculation method of Dragsted and Lang gave a LD50 value of 5.044 ± 0.28 g/kg b.w. There is no significant difference between the two values of LD_{50} ($p>0.05$).

Table 2: Acute toxicity of BFBu by intraperitoneal administration in mice

Groups of 10 mice	Dose of BFBu (g/kg b.w.)	Number of mice dead	Mortality %
1	NS (Control)	0	0
2	1	0	0
3	2	2	20
4	4.065	4	40
5	6.024	6	60
6	10	8	80
7	12.048	10	100

Dose response effects of BFBu on the electrocardiogram of rabbit

As shown in table 3, the dose response effect of BFBu was achieved on rabbit electrocardiogram. BFBu affected the different parameters of the electrocardiogram of rabbit. Indeed, for doses ranging from 0.5 to 40 mg/kg b.w., the control values of the amplitude of P (149 ± 21 µv), QRS (813 ± 103 µv) and T (157 ± 20.9 µv) waves were dose dependently reduced and attained 78.4 ± 8.38 for P, 368 ± 34.1 for QRS and 52.5 ± 8.44 for T waves at the dose of 40 mg/kg b.w. The cardiac frequency also significantly ($p<0.05$) and dose dependently dropped from 221 ± 6.91 cycles/min (Control value) to 116 ± 11.9 cycles/min at the dose of 40 mg/kg b.w. PQ interval slightly and not significantly decreased from 0.5 to 5 mg/kg b.w. The control value (83.6 ± 4.12 ms) reached 79.6 ± 0.4 ms (0.5 mg/kg b.w.), 81.2 ± 1.74 ms (2.5 mg/kg b.w.) and 80.8 ± 1.36 ms (5 mg/kg b.w.). From 10 mg/kg b.w. to 40 mg/kg b.w., PQ returned to the control value. So, in this range of doses, PQ was not affected. However, QT interval was significantly ($p<0.05$) and dose dependently augmented from 155 ± 4.76 ms (Control value) to 287 ± 8.45 ms at 40 mg/Kg b.w.

Table 3: **Dose-response effect of BFBu on rabbit ECG**

Doses of BFBu (mg/kg b.w.)	Amplitudes (µv)			Intervals (ms)		Frequency (cycles/min)
	P	QRS	T	PQ	QT	
0 (Control)	149±21	813±103	157±20.9	83.6±4.12	155±4.76	221±6.91
0.5	144±20.3	784±102	151±20.8	79.6±0.4	156±3.85	213±6.39
2.5	137±18.1	767 ±102	144±21	81.2±1.74	176±3.85	202±6.05
5	130±18.1	638±91.9	131±21	80.8±1.36	187±3.38	190±6.50
10	120±18.1	615±89.9	110±14	83.6±4.12	197±3.38**	179±7*
20	100±15.8	521±80.1	82.8±10.2	83.6±4.12	208±3.66***	143±12.6***
30	95±15.8	421±55.6	59.7±7.25**	83.6±4.12	232±5.83***	135±10.4***
40	78.4±8.38	368±34.1*	52.5±8.44**	83.6±4.12	287±8.45***	116±11.9***

Effects of BFBu in presence of atropine on the electrocardiogram of rabbit

The effects of BFBu were investigated in presence of atropine (Table 4). BFBu at 30 mg/kg b.w. elicited decrease of P (30.60 ± 6.15%), QRS (42.90±4.69%), T (58.50 ± 3.16%) waves and cardiac frequency (34 ± 5.01%) and increase of QT interval (49.90 ± 2.87%). PQ interval was not affected. These values were control values of the effects of BFBu at 30 mg/kg b.w. In the presence of increasing doses of atropine (5.10^{-8} to 5.10^{-2} mg/kg b.w.), the effects induced by BFBu at 30 mg/kg b.w. were significantly ($p<0.05$) and dose dependently attenuated by atropine. Indeed, P, QRS and T waves impaired decreases and reached 10.40 ± 1.76%, 10.90 ± 4.08% and 12 ± 3.05% respectively in the presence of atropine at 5.10^{-2} mg/kg b.w. Cardiac frequency also decreased to attain 8.17 ± 1.10% in presence of atropine at the dose of 5.10^{-2} mg/kg b.w. QT interval was found to produce an increase of 13.80 ± 1.23% in presence of the same dose of atropine.

Table 4: **Dose-response effect of BFBu in presence of atropine on rabbit ECG**

Doses of BFBu and atropine (mg/kg b.w.)	Amplitudes (%)			Interval (%)	Decrease of Frequency (%)
	Decrease of P	Decrease of QRS	Decrease of T	Increase of QT	
BFBu 30	30.60±6.15	42.90±4.69	58.50±3.16	49.90±2.87	34.00±5.01
Atr 5.10^{-8}	26.20±5.90	34.30±3.32	45.30±2.70*	34.40±2.24***	30.10±5.61
Atr 5.10^{-6}	18.10±3.58	23.90±3.09*	31.30±2.57***	27.40±2.14***	18.30±2.62*
Atr 5.10^{-4}	13.30±2.51	20.90±3.13**	21.60±3.74***	21.00±1.92***	13.70±2.08**

Atr 5.10^{-2}	10.40±1.76*	10.90±4.08***	12.00±3.05***	13.80±1.23***	8.17±1.10***

Phytochemical screening

As shown in Table 5, the phytochemical analyses of the butanol extract of the leaves of *Blighia unijugata* revealed the presence of sterols and polyterpenes, polyphenols, flavonoids and saponins.

Table 5: Phytochemical screening of the butanol fraction of *Blighia unijugata* (BFBu).

chemicals constituents	BFBu
sterols and polyterpenes	+
polyphenols	+
reduced sugar	-
flavonoids	+
saponins	+
tannins	-
coumarines	-
quinones	-
alkaloids Dragendorff	-
alkaloids Burchard	-
cardiotonic glycosides	-

DISCUSSION

The study of the acute toxicity of the butanol fraction of *Blighia unijugata* (BFBu) in mice permitted to determine LD_{50} by oral and intraperitoneal ways. The graphic method of Miller and Tainter gave 20.14 ± 0.44 g/kg b.w. and 5.628 ± 0.29 g/kg b.w. respectively for oral and intraperitoneal administrations. With the calculation method of Dragsted and Lang, 21 ± 0.54 g/kg b.w. and 5.044 ± 0.28 g/kg b.w. were obtained respectively for oral and intraperitoneal administrations. The values of LD_{50} obtained graphically and by calculation methods were quite similar by oral administration. The same observation was noticed with intraperitoneal administration of BFBu. According to the classification described by an author [22], BFBu is neither toxic by oral way nor by intraperitoneal injection. Therefore, the leaves of this plant could be considered as safe for its traditional use by populations. However, additional tests (subchronic and chronic toxicity) are required to guarantee the complete safety of its use. The acute toxicity of a substance can be different according to the dose and the way of administration [23]. This was shown with plant extracts such as the aqueous leaf extract of *Piliostigma reticulatum* (Caesalpiniaceae) which LD_{50} was equal to 17 g/kg b.w. orally and 1.5 g/kg b.w. by intraperitoneal way [24], and the aqueous extract of *Bridelia ferruginea* Benth. (Euphorbiaceae) with 3.14 ± 0.58 g/kg b.w. and 0.11 ± 0.029 g/kg b.w. as LD_{50} respectively by oral and intraperitoneal ways [25]. The LD50 obtained by oral administration of BFBu is higher than those of other plant extracts like the aqueous extract from the stem barks of *Pterocarpus soyauxii* Taub. (Papilionaceae) with a DL_{50} of 10.75 g/kg b.w. and the aqueous extract from the stem barks of *Terminalia superba* (Combretaceae) with a LD50 of 12.33 ± 0.87 g/kg b.w. [26, 27]. BFBu is not toxic by intraperitoneal way as compared to extracts such as a chromatographic fraction of *Bidens pilosa* leaves which exhibited moderate toxicity with a LD_{50} value of 452.50 ± 23.10 mg/kg b.w. [19].

The effects of the butanol fraction of *Blighia unijugata* were investigated on the electrocardiogram of rabbits. BFBu induced decreases of P, QRS, T waves and cardiac frequency. QT interval was significantly augmented and PQ interval was not affected. These results revealed that BFBu could decrease the depolarization of the atria and ventricles, ventricular repolarization and heart rate. Atropine, a muscarinic cholinoceptors, significantly inhibited the effects elicited by BFBu. These results suggested the presence of cholinomimetic substances in the butanol extract of *Blighia unijugata.* These compounds could be

responsible for the inhibitory activities of BFBu on the global electrical activity of the heart of rabbit. Indeed, the actions of acetylcholine on the global electrical activity of heart were shown by some researchers on a heart-lung preparation of dog [28]. According to these authors, acetylcholine triggered a slowing of the pacemaker, a blockade of atrio-ventricular conduction associated with prolongation of PR interval and a decrease in ventricular activity. These findings were similar to those of two groups of researchers who showed respectively that the aqueous extract of *Mirabilis jalapa* at doses ranging from $4.27x10^{-4}$ to $1.34x10^{-2}$ g/kg b.w. and BpF_2, an active chromatographic fraction from *Bidens pilosa* (5-25 mg/kg b.w.) caused decreases in the amplitudes of P, QRS, T waves and heart rate of rabbits [19, 29]. However, while PQ interval was not affected with BFBu, the aqueous extract of *Mirabilis jalapa* and BpF_2 revealed a decrease and an increase of PQ interval respectively. Some authors also highlighted a cholinergic effect of the aqueous extract of *Sesamum radiatum* on rabbit electrocardiogram by achieving the interaction of their extract with atropine [17]. QT interval was significantly increased. This result suggested that BFBu could be source of arrhythmias since according to some scientific workers, a prolongation of PQ interval could lead to such outcomes [30, 31].

The phytochemical screening carried out for BFBu indicated the presence of sterols and polyterpenes, polyphenols, flavonoids and saponins. The cardioinhibitory effects of BFBu could be due to polyphenols and flavonoids in the extract. Indeed, these compounds are recognized to produce beneficial effects on the cardiovascular system. Some researchers showed this on experimental animals [32-34]. Moreover, other authors pointed out the extensive beneficial role of polyphenols on the cardiovascular system [35].

CONCLUSION

The acute toxicity of the butanol fraction from the leaves of *Blighia unijugata* was assessed and revealed that this extract was not toxic when administered orally and intraperitoneally. However, sub-acute and chronic toxicity must be implemented to assure a complete safe use of this plant. The investigations carried out on the electrocardiogram of rabbit exhibited a decrease of different waves while QT interval increased and PQ interval was not affected. The partial inhibition of these effects by atropine suggested the presence of cholinomimetic substances in the extract. The heterogeneity of chemical compounds particularly polyphenols and flavonoids could explain the effects of this extract on the cardiac activity and therefore justify the use of this plant in traditional medicine to treat cardiovascular disease.

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