ANTIMICROBIAL POTENCY AND REVERSION OF THE BACTERIAL RESISTANCE OF ETHANOLIC EXTRACT OF *MOMORDICA CHARANTIA* LINN.

Houéto E.E.M.[1*], R.C. Johnson, A.M.O. Amoussa[2], H.W. Kpètèhoto[1], F.M.Z. Mignanwandé[1], F. Loko[3] and L. Lagnika[2]

[1]*Laboratory of Hygiene, Sanitation, Toxicology and Environmental Health, Interfaculty Center of Training and Research in Environment for the sustainable Development, University of Abomey-Calavi (UAC), 01 PO Box 1463, Cotonou, Benin.*
[2]*Laboratory of Biochemistry and Natural Bioactives substances, Faculty of Sciences and Technology, University of Abomey-Calavi, Bénin.*
[3]*Laboratoire de Recherche en Biologie Appliquée (LARBA) à l'École Polytechnique d'Abomey-Calavi (EPAC) de l'Université d'Abomey-Calavi (UAC / Bénin).*

ABSTRACT

Momordica charantia L., a plant of the family cucurbitaceae, commonly used in Benin, is known for its antimicrobial potency. This research evaluates the biological properties of *M. charantia* for safe use. The material used consisted of eight bacterials, ethanolic extract of *Momordica charantia* and female wistar strain rats. Minimum inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) were investigated by microdilution and plating, kinetics of action by spectrophotometry, and reversion of bacterial resistance by microdilution. The Traces Metallic Elements (ETM) were assayed by Metalyser protocol. The safety tests were performed according to the Organisation for Economic Co-operation and Development (OECD), Standards 423 (Acute Toxicity) and 407 (Subchronic Toxicity) guidelines and the blood sample by retro-orbital sinus puncture. The significance of the statistical results was set at $P < 0.05\%$. The results reveal 90% of tested germs sensitive to the extract of *M. charantia* whose search for MIC and MBC shows a bactericidal effect on 57.14% and bacteriostatic on 42.86%. The kinetics of action reveals an antibacterial effect between 8 to 16 hours in contact with germs. The conventional antibiotic-extract combination reveals a significant change in MICs with decreases of up to 90%. Acute toxicity has not shown significant modification of the cellular structures on the histological sections. *M. charantia* has antibacterial properties and its punctual use is safe at the doses tested.

Keywords : *Momordica charantia*, Extract, Toxicity, biological activity; reversion, Safety.

INTRODUCTION

Of the many antibiotics, beta-lactams are currently the most widely used in the world, especially in developing countries (Toty *et al*., 2013). However, due to its anarchic, inadequate and abusive use in human and veterinary health, we are now witnessing the emergence of multi-resistant bacteria (Savard *et al*., 2003). This is becoming a very serious public health problem (Lozniewski *et al*., 2010). It is, therefore, important to look antibacterial principles. There is also the problem of contamination by heavy metals. These are major contaminants in the environment that pose serious ecological problems, both by their ubiquitous nature in the biosphere with high toxicity (Boyd, 2010).

The aim of the study was, therefore, to test the antimicrobial properties of *Momordica charantia* and to evaluate the possible effects of its use on the vital organs such as the liver and kidney. The effects of toxicities due to herbal medicine can occur in a number of organs such as the kidneys, liver, stomach, nervous system and blood (Ogbe *et al*., 2012 ; Asiimwe *et al*., 2014) .

MATERIALS AND METHODS

Collection of plant Material:

Authenticated at the national herbarium at the University of Abomey Calavi, under the number AA 6723 / HNB, this plant material was harvested in Avakpa, a village in the commune of Allada located at 56 km from Cotonou in Republic of Benin.

***Corresponding authors**: Eustache Enock Meinsan HOUETO, 01 PO Box 1778, Cotonou, Benin.*
Email : houeto.eustache@gmail.com; Phone : (00229) 94 448 113 /97 878 054

Preparation of the ethanolic extract

The dried plant was reduced to powder. 100 g of powder obtained were brought into contact with the solvent with mechanical stirring for 72 hours. The extract was decanted, filtered and evaporated before being stored in 4 ° C. containers.

Bacterial strains

The microorganisms used in this study are reference strains (Table 1) and germs isolated from biological samples in the microbiology laboratory of the National University Hospital Center/ Hubert Koutoukou Manga (CNHU / HKM) (Table 2). These germs were preserved by transplanting on agar medium.

Animal material

Wistar strain rats, 12-week old non-pregnant nulliparous females (for acute toxicity according to OECD 423 standards) and both sexes (for sub-chronic toxicity, according to OECD 407 standards), with a mass of between 160 and 200 g, were used.

Sensitivity Test

The sensitivity test was performed using a Muller Hinton (MH) gelose diffusion method to test the antibacterial activity of the extract. All cultures were made in triplicate at the incubation temperature. . An extract of 10 mg / mL was used with a 106UFC concentration inoculum. Sterile wattman paper (Qualitative Circles 150 mm cat # 1001-150) was impregnated for testing and commercially available antibiotic discs (Bio Mérieux) of ciprofloxacin and ceftriaxone served as controls. 18 to 24 hours later, the observation of the boxes was made.

Determination of Minimal Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentrations (MBC)

The MIC was determined using a microdilution method recommended by NCCLS M7-A6 (Amoussa *et al.*, 2016). Before carrying out the tests, the bacterial strains sensitive to the antibacterial power of the extract were diluted in MH broth and incubated for 18 h at 37 ° C, and the suspension was brought to a concentration of 1 × 10 6 CFU / mL. An extract concentration range of 10 mg / mL to 0.078 mg / mL was used.

An acetone-water mixture and gentamicin were used as a negative and positive control. All experiments were performed in triplet and microdilution plates were incubated at 37 ° C for 24 h. Antibacterial activity was detected using a colorimetric method by adding 25 µL of 0.01% aqueous Iodonitrotetrazolium (INT) solution to each well at the end of the incubation period. The plate is then incubated at 37 ° C for 30 minutes. The method of determination of the MIC was coupled to the spreading on solid medium, according to the technique of Chabert and Daguet (1985) adapted to the conditions of the LBSNB, for the determination of the MBC. The MBC was determined by spreading 10 µL of the contents of each tube of concentration greater than or equal to the MIC on a solid medium. After incubation, the concentration at which there is no visible growth on the agar plate was recorded as the minimum bactericidal concentration. The antibacterial effect will be considered bactericidal or bacteriostatic depending on the ratio: MBC / MIC

According to the method of Fauchere and Avril (2002), for which a substance is bactericidal when the ratio MBC / MIC ≤ 4, and bacteriostatic when this ratio is> 4.

Determination of the bactericidal action kinetics of the extract

The suspensions used were fresh cultures of 24 hours of concentration 10^6 CFU. The extracted inoculum mixture was made volume-to-volume in sterile tubes, and incubated at 37°C. The optical densities were read at T0, then at intervals of 4 hours up to 36 hours, against a blank consisting of a mixture of MH and extract diluted at the concentrations of the various tests. Curves were plotted on the basis of turbidity variance over the duration of the experiment.

Evaluation of the reversion of bacterial resistance

The main objective of this test was to determine a possible synergy of action between the extract and the conventional antibiotic. It consists of looking for the MICs of conventional antibiotics and those of the combination of antibiotics and extract in order to calculate Inhibitory Fractional Concentration (IFC). $\frac{\text{MIC (combination)}}{MIC\ (ATB)}$

According to the Antibiogram Committee of the French Society of Microbiology and reported by Bassolé and Juliani (2012), there is:

- Synergy if CFI ≤ 0.5

- Addition if $0.5 < CFI \leq 1$
- Indifference if $1 < CFI \leq 4$
- Antagonism if $CFI > 4$

For this, five antibiotics, [Amoxicillin (AMX), Ciprofloxacin (CIP), Ampicillin (AMP), Erythromycin (ERY), Cotrimoxazole (SXT)] and two germs E. coli [because of its prevalence in infections, Jarlier *et al*., 2012)] and P. aeruginosa [because of its multidrug resistance (Li *et al.,* 2012)] were chosen.

- 50 µL of the antibiotic solution of 1 mg were distributed in the first and second wells of the microplate. 50 µL of the solution of MH were distributed in the wells from the second
- A cascade dilution was then made until the end.
- 50 µL of extract are added to each well at MICs of the different seeds.

100 µL of the inoculum were added to all the wells.

- The incubation at 37 ° C lasted 24 hours.
- The reading was made after adding 40 µL of INT at 0.01%.
- A second incubation was done for 30 minutes.
- The presence of germ results in the appearance of a red color of the medium.

Controls without plant extract, or without bacterial suspension or without antibiotics were made.

Acute toxicity

Two groups of eight female rats were used. The first group serves as a control while the second receives a dose of 5000 mg / extract per kg of weight. The animals were weighed and the blood samples taken before the rats sacrificed. The acute oral toxicity study was conducted according to the procedure described by method 423 (OECD, 2001).

Subchronic toxicity

The subchronic toxicity study was conducted according to OECD standards 407 (OECD, 2008). The four groups of rats were designated A, B, C, and D. Group A was used as a control while B, C and D received respectively 500, 750 and 1000 mg of ethanolic extract / kg of body weight daily for 28 days. The animals were weighed on the 1st, 7th, 14th, 21st and 28th days. Blood samples were taken on the 28th day before the animals were sacrificed. Consumption of food and water were evaluated. In both types of toxicity, the biological parameters analyzed were creatinine, urea and transaminases.

Determination of Traces Metallic Elements (ETM)

The dosage of the ETM was made with a portable analyzer, heavy metals (HM 3000 Metalyzer). This technique was based on the method of voltammetry which consists of a pre-concentration of metals on the surface of the electrodes followed by a scanning of the potential to dissolve the deposited metals, allowing a quantification of this metal (Lagnika *et al.*, 2016). In this study, arsenic (As), lead (Pb), cadmium (Cd), mercury (Hg) were assayed.

Statistic study

The data was entered in Excel 2013 ($P < 0.05$). The t-student test was used for the statistical analysis of data on body weight, hematological and biochemical parameters. The difference was considered statistically significant when the p-value was less than 0.05 ($p < 0.05$). All data were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Of the eight organisms selected for our study, seven were sensitive to the *M. charantia* extract (Table 3) according to the reading scale (Ouattara *et al.*, 2017). According to several authors (Kumar *et al.*, 2010; Johnson *et al.*, 2016; Jobi *et al.*, 2017), *Momordica charantia* is a plant with known antibacterial activity.The determination of the MICs, CMBs and their different interpretations were made (Table 4). After the test carried out on the seven remaining germs, the extract had a bactericidal effect on four germs and bacteriostatic out of three. These results corroborate those of some authors who find that:

- the fresh and dried leaves of *Momordica charantia* showed significant antimicrobial activity through the ethyl acetate fractions, effective against *Escherichia coli* and *Bacillus cereus* (Galberto *et al.*, 2010).
- The aqueous extracts of *Momordica charantia* leaves are more active on *Escherichia coli*, *Salmonella sp*. and *Staphylococcus aureus* with percent inhibition of 92.64, 91.86 and 93.57% respectively (Gbogbo *et al*., 2013).

Other authors confirm these results but they used other solvents (petroleum ether and methanol) on germs such as K. *pneumoniae*, S. *aureus*, C. *albicans* and S. *typhi* (Mwambete, 2009). According to them, the antibacterial

activity of the ethanolic extract is more interesting than that of the aqueous extract. This can be attributed to the presence of soluble phenols and polyphenolic compounds that are readily extracted from non-polar solvents such as ethanol as compared to polar solvents such as water (Mada et *al*., 2013) (Gbogbo *et al.,* 2013). The evaluation of these different activities can be seen through Minimal Inhibitory and Bactericidal Concentrations.

MICs range from 125 µg to 625 µg / ml for the tested microorganisms. As for CMB, they vary between 1250 and 5000 µg / mL. They made it possible to characterize the effect of the extract on the different germs. In comparison with other plants known for their antibacterial power (Arani *et al.,* 2011),

The action of the extract on the germs is done according to a certain dynamic that is interesting to know. The general analysis of the curves resulting from the action kinetics of the extract shows:

- The general analysis of the curves resulting from the action kinetics of the extract shows:

- A first phase (from 0 to 8h) of growth of germs at all inocula, but stronger at the level of inucula containing extracts compared to bacterial broths. The good growth parameter is explained by the presence of the primary metabolites provided by the M. charantia extract, which make the culture medium richer in nutrients (Krishnendu *et al.,* 2016, Johnson *et al*., 2016).

- A second phase of decay abhors the curve. The duration of this step is different from one seed to another. From 8 to 16 h for MRSA and S. aureus, from 8 to 20 h for E. *faecalis* it is 8 to 36 h for E. coli isolated from biological fluid. This step reflects a decrease in the concentration of germs in the medium for the extracted inoculum mixture. Meanwhile, the concentration of bacterial broth continues to increase. The antibacterial effect of the extract would have begun its effect.

- The third phase, ranging from 16h to 36h for MRSA and S. *aureus*, and from 20h to 36h for E. *faecalis* which is the phase of absence of any significant variation in the medium. At this stage, the germs of the medium have already had their growth inhibited; and it is towards the end of this phase that a stability was observed at the level of bacterial broths without extract. This would translate a saturation into germ of the culture medium.

The same phases were observed for *Piper betle*, a plant native to Asia. With *Pseudomonas aeruginosa*, for an extract concentration of 100 µg / ml, the decline phase was observed after 4h (Arani *et al.,* 2011). The low MIC of the action of its extract on the different germs explains its greater antibacterial power.

16 hours after the start of the experiment, the growth of MRSA and *S. aureus* were inhibited. That of *E. faecalis* was only 20 hours later. As for isolated *E. coli*, it is only after 36 hours that his is inhibited. The relative efficacy of the bactericidal activity of the extract of M. charantia on the various germs in study, pushes to continue research while associating the use of the plants with that of the conventional antibiotics.

E. *coli* is sensitive to the effect of Amoxicillin at a concentration of 1000 µg and ciprofloxacin at 250 µg. In addition, no other product tested had any effect on the germ. After adding the extract of *Momordica charantia* to antibiotics, MICs decreased by 1000 to 125 µg and 250 to 7.8 µg / mL (the lowest tested rate) respectively for Amoxicillin and Ciprofloxacin. As for the other antibiotics, to which the germ was not sensitive, MICs of 500, 125, and 250 µg / mL were obtained respectively for ampicillin, erythromycin and cotrimoxazole. The calculated CFIs showed a synergistic action between the extract and the different ATBs used in this experiment. There was therefore a reversal of bacterial resistance in all cases for *E. coli* (Table 5,6 and 7).

For P. *aeruginosa*, the sensitivity is only observed for ciprofloxacin at a MIC of 500 µg / ml. At the addition of the extract at 625µg / mL, *P. aeruginosa* is sensitive to all other antibiotic except for amoxicillin and ampicillin. As for ciprofloxacin, the MIC went from 500 to 31.2 µg/mL, a decrease of more than 90%. Antibiotics, which had no effect, could, in combination with the extract, inhibit bacterial life up to MICs of 250 and 500 µg / mL, respectively for Erythromycin and cotrimoxazole. This denotes the effectiveness of the combination thus showing a synergy in the effect of the two products. For amoxicillin and ampicillin, no sensitivity was detected before or after combination.

This persistent resistance could be explained in two ways. *P. aeruginosa* is a germ that is naturally resistant to many antibiotics. It has the ability to acquire very quickly other types of resistance either by enzymatic mechanism (production of proteases or beta-lactamases) or by non-enzymatic mechanism (impermeability) (Chaibdraa *et al.,* 2008).

Fractional Inhibitory Concentrations have shown that the combined action of the extract and conventional ATBs on *E. coli* is synergistic. When with *P. aeruginosa*, the combined actions of the extract - ciprofloxacin and extract - cotrimoxazole synergistic. There is therefore a reversal of bacterial resistance with all the germs used in this study except *P. aeruginosa* in combination with betalactamines. These results corroborate those of Khode *et al.* (2017) who found that the ethanolic extract had better antimicrobial activity in combination with Cefotaxime and Ceftriaxone, against *E. coli* and. *S. aureus* as the aqueous extract.

Table 1. Reference strain of bacteria.

Family	Genus / Species	Reference	Types
Staphylococcaceae	*Staphylococcus aureus*	ATCC 6538	Positif
	Staphylococcus Aureus Resistant to Meticillin		
	Staphylococcus epidermidis	CIP 8039	
Enterococcaceae	*Enterococcus faecalis*	ATCC 292012	
Enterobacteriaceae	*Escherichia coli*	CIP 53126	Négatif
Pseudomonadaceae	*Pseudomonas aeruginosa*	CIP 82118	

Table 2. Hospital strains employed in the study.

Family	Genus /Species	Origin	Gram
Enterobacteriaceae	*Escherichia coli*	Urine	
Pseudomonadaceae	*Pseudomonas aeruginosa*	Pus	Negative

Table 3. Sensitivity Test Results.

Microorganisms	*M. charantia* (Diameters in mm)			mean ± STDEV	Sensitivity of germs
	1st test	2st test	3st test		
E. coli isolated	9	10	9	9,33±0,57	+
E. coli CIP 53126	8	9	9	8,66±0,57	+
P. aeruginosa isolated	11	10	11	10,66±0,57	+
P. aeruginosa CIP 82118	10	9	9	9,33±0,57	+
MRSA	10	9	9	9,33±0,57	+
E. faecalis ATCC 29212	8	9	8	8,33±0,57	+
S. aureus ATCC 6538	14	14	13	13,66±0,57	+
S. épidermidis CIP 8039	7	6	6	6,33±0,57	-

+ , Sensitive germs eligible for IMC research ; **-** , Resistants germs not eligible IMC research.

Table 4. Results of the MICs and MBC

Bacterial strains	Ethanolic extract of *M. Charantia*		Result and interpretation	
	MIC (µg.ml^{-1})	MBC (µg.ml^{1})	MBC/MIC	interpretation
E. coli isolated	312	1250	4.0	bactericidal
E. coli CIP 53126	312	2500	8.0	bacteriostatic
P. aeruginosa isolated	625	5000	8.0	bacteriostatic
P. aeruginosa CIP 82118	312	5000	16	bacteriostatic
MRSA	625	2500	4.0	bactericidal
E. faecalis ATCC 29212	125	250	2.0	bactericidal
S. aureus ATCC 6538	125	250	2.0	bactericidal

Table 5**.** Results of MICs of ATBs

		Esherichia coli					*Pseudomonas aeruginosa*				
	ATB	AMX	CIP	AMP	ERY	SXT	AMX	CIP	AMP	ERY	SXT
Different doses of ATB	1000	+	+	-	-	-	-	+	-	-	-
	500	-	+	-	-	-	-	+	-	-	-
	250	-	+	-	-	-	-	-	-	-	-
	125	-	-	-	-	-	-	-	-	-	-
	62.5	-	-	-	-	-	-	-	-	-	-
	31.2	-	-	-	-	-	-	-	-	-	-
	15.6	-	-	-	-	-	-	-	-	-	-
	7.8	-	-	-	-	-	-	-	-	-	-

+ : Inhibition of bacterial life; **-** : Absence of inhibition of bacterial life

Table 6**.** MIC Results of ATB Combinations – Extracts

ATB-Extract		*Escherichia coli*					Ext.	*Pseudomonas aeruginosa*				
Ext.	ATB	AMX	CIP	AMP	ERY	SXT		AMX	CIP	AMP	ERY	SXT
extract at 312 µg/ml	1000	+	+	+	+	+	extract at 625 µg/ml	-	+	-	+	+
	500	+	+	+	+	+		-	+	-	+	+
	250	+	+	-	+	+		-	+	-	+	-
	125	+	+	-	+	-		-	+	-	-	-
	62,5	-	+	-	-	-		-	+	-	-	-
	31,2	-	+	-	-	-		-	+	-	-	-
	15,6	-	+	-	-	-		-	-	-	-	-
	7,8	-	+	-	-	-		-	-	-	-	-

+ : Inhibition of bacterial life; **-** : Absence of inhibition of bacterial life; Ext. = Extract

Table 7. Inhibitory Fractional Concentrations

Germs	AMX	CIP	AMP	SXT
Escherichia coli	0.125	0.0312	< 05	< 0.25
Pseudomonas aeruginosa	Undetermined	0.0624	undetermined	< 0.5

Table 8**.** Variation of the Masses of the of materials consumed (acute toxicity)

Material consumption	Weeks	Quantity of materials consumed	
		Control	Single dose 5000 mg/Kg
Granules (g)	1	80,71±7,01	73,28 ± 8,75
	2	78,71±5,99	79,14 ± 7,023
P		0,1844154	
Water (l)	1	99,28±19,02	96,42 ± 17,25
	2	124,28±6,72	122,14 ± 11,12
P		0,7317415	

Similar phenomena have been observed by other authors. A reversal of the bacterial resistance of *Staphylococcus aureus* and *Klebsiella pneumoniae* was observed with ethanolic extract of *Syzygium aromaticum* and antibiotics such as imipenem, cotrimoxazole, levofloxacin, amoxicillin + clavulanic acid, and erythromycin (Atteia and Hussein, 2014).

These results are also similar to those of some researchers who, by combining vancomycin with pineapple extract, succeeded in transferring the bacterial resistance of *Steptococcus sanguis* by moving the MIC of vancomycin from 1 µg to 0.5 µg / mL. (Emami *et al.*, 2013). Many studies have shown a significant reduction in MICs of antibiotics, when combined with plant extracts (Betoni *et al.*, 2006, Al-hebshi *et al.*, 2006, Atteia *et al.*, 2014). All this could be explained by the fact that some secondary metabolites identified in the plant extracts, have

the possibility of inactivating the modified targets of the antibiotics thus causing a sensitivity of the germ, and thus a reversal of their resistance (Hemaiswarya *et al.*, 2008).

The possible negative effects of this plant on human health must therefore be evaluated. During the experiment, animal monitoring revealed the following: No deaths were observed during the experimental period. The extract is not lethal. These results are consistent with those of Salawu *et al.* (2004) and Vikram and Sandeep (2015). The acute toxicity of *Momordica charantia* decocts is practically nil (Senghor, 1994). All experimental rats survived until the end of the treatment period. The acute oral toxicity test performed on sprague dawley strain rats showed that the ethanolic extract of *M. charantia* at a dose of 2000 mg / kg had no adverse effect on them. (Laleye *et al.*, 2015) These results are also consistent with those of Narendra *et al.* (2016) but are contradicted by those of Abalaka *et al.* (2009) who noted the death of all rats fed with an extract of 1500 mg / kg of body weight. These contradictions can be generated by climatic and environmental pedological factors of the places of harvest.

Table 9.Variation in the quantity of materials consumed (subchronic toxicity).

Material consumption	Weeks	Quantity of materials consumed			
		Control	Different batches of rats		
			500 mg / Kg	750 mg / Kg	1000 mg / Kg
Granules (g)	1	133,25±21,01	136,42± 21,13	108,62±62,28	113,57±32,52
	2	131,28±11,87	118,57±29,63	105±34,65	107,57±33,09
	3	138,42±16,58	133,71±16,84	105,28±13,64	102,57±12,31
	4	135,28±9,39	136,28± 15,93	102,71±8,24	99,71±14,99
	Monthly average	134,71±14,73	131,25±21,69	105,32±34,48*	105,85±24,30*
Water (l)	1	282,14±36,38	288,57±48,27	225 ±56,05	256,42±46,07
	2	310±43,87	345,28 ±84,18	235±16,32	238,57±27,49
	3	250±40,20	300±29,01	250±13,22	255,71±13,97
	4	281,42±25,93	332±23,97	256,14±20,47	265±30,68
	Monthly average	280,89±41,2	316,46±54,38*	243,78±33,61*	253,92±31,42*

Table 10. Evolution of body mass of animals.

Days	Mass of the different lots(g)	
	Sample batch	Test batch
J_1	191±2,51	177,33±7,57
J_7	190±1,52	188,66±3,05
J_{14}	193±66±2,08	204,33±13,05*

Table 11. Results of changes in body mass of animals.

Days	Body mass of rats (g ± SD / SEM)			
	Control	Different dose of extract		
		500 mg / Kg	750 mg / Kg	1000 mg / Kg
J_1	186,12±8,09	154,5± 19,88	166,62±15,29	183,25±23,63
J_7	188,87±5,38	160,62±25,39	167,12±12,31	197,62±20,13
J_{14}	194±7,32	169,12±19,30	168,12±16,50	206,87±20,67*
J_{21}	197,5±6,11	185±27,63	182,62±10,34*	214,75±19,78*
J_{28}	201,87±6,08	215,75±30,14*	207,37±33,91*	207,37±33,91*

Table 12. Result of the determination of hematological parameters (acute toxicity)

hematological parameters	Lot test	Sample batch	P value
RBC	6,95 ± 0,48	7,64± 0,47	0,16219622
WBC	6,66 ± 2,1	9,325±0,85	0,06519448
Hb	13,53 ± 0,73	13,43±1,62	0,68610667
Ht	39,93 ±2,85	42,56± 2,07	0,26918564
Plt	468,33 ± 83,34466	726,75±182,3	0,06938679

Table 13. Result of the determination of hematological parameters (subchronic toxicity)

Parameters	Control	Different batches of rats		
		500 mg/Kg	750 mg/Kg	1000 mg/Kg
WBC (10^3 / mm^3)	9,47±0,77	9,6± 2,24	6,07±2,35*	7,15±3,07
RBC (10^8 / mm^3)	7,56±0,37	7,1± 0,51	6,63±0,78*	6,59±1,69
Hb (g / dl)	12,92±1,66	13,88±0,86	12,81±1,07*	12,45±2,99
Ht (%)	42,7±2,3	40,8±2,69	38,77±3,46*	37,66±9,05
PLt (10^3 / mm^3)	748,5±161	678,35±83,58	711±304	540,12±160,34*
MCHC (%)	30,2±3,35	34,01±1,06	32,82±0,68	32,45±1,25
MCH (pg/mm^3)	17,05±1,85	19,37±0,68	19,37±0,85	18,95±1,38
MCV (μm^3)	56,47±0,23	56,92±2,19	58,65±2,89	49,94±17,69

Results are mean ±S.E.M., significantly different from control: P< 0.05
Key: WBC= white blood cells, RBC= red blood cells, Hb=haemoglobin, Ht =hematocrit, MCV=mean cell volume, MCH=mean cell haemoglobin, MCHC=mean cell haemoglobin concentration, PLT=platelets.

Table 14. Result of the assay of biochemical parameters (acute toxicity)

Different batches	BUN	CRE	AST	ALT
Control batch	0,33 ±0,04	6,42±0,38	182,52 ±21.04	95,04 ±13,89
Test batch	0,64±0,075*	5,7± 0,360	189,23±35,990	100,43±18,650

Results are mean ± S.E.M., significantly different from control: P< 0.05
Key: BUN=urea, CRE=creatinine, AST=asparate aminotransferase, ALT=alanine aminotransferase.

Table 15. Result of the assay of biochemical parameters (subchronic toxicity)

Parameters	Control	Different batches of rats		
		500 mg / Kg	750 mg / Kg	1000 mg / Kg
BUN (g / L)	0,33 ±0,03	0,49± 0,044*	0,60±0,11*	0,61±0,08*
CRE (mg/L)	6,33±0,30	4,725± 0,64*	6,36±1,88	6,27±0,43
AST (UI/L)	183,27±14,43	175,23 ± 22,34	164,16±36,53	185±18,96
ALT(UI/L)	91,84 ±9,78	149±138,53*	113,55±12,60*	105,45±18,84*

Table 16. Result of the variation of the masses of the organs of the animals

Organs	weights of organs (g ± SD / SEM)			
	Control	Different batches of rats		
		500 mg/Kg	750 mg/Kg	1000 mg/Kg
Liver	5,46±0,30	6,93±0,49	6,86±1,18	6,43±1,06

Table 17. Les concentrations Fractionnaires Inhibitrices

	AMX	CIP	AMP	ERY	SXT
E. coli	0.125	0.0312	< 0.5	< 0.5	< 0.25
P. aeruginosa	indéterminé	0.0624	Indéterminé	< 0.5	< 0.5

Table 18. Results of the ETM assay.

Requested ETM	Results (ppb)	Results (ppm)	WHO standards (ppm)
Arsenate (AsIII)	0,83	$0{,}83.10^{-3}$	≤ 1
Mercury (Hg)	Undetermined	Undetermined	≤ 0,1
Lead (Pb)	0,46	$0{,}46.10^{-3}$	≤ 0,3
Cadmium (Cd)	Undetermined	Undetermined	≤ 0,2

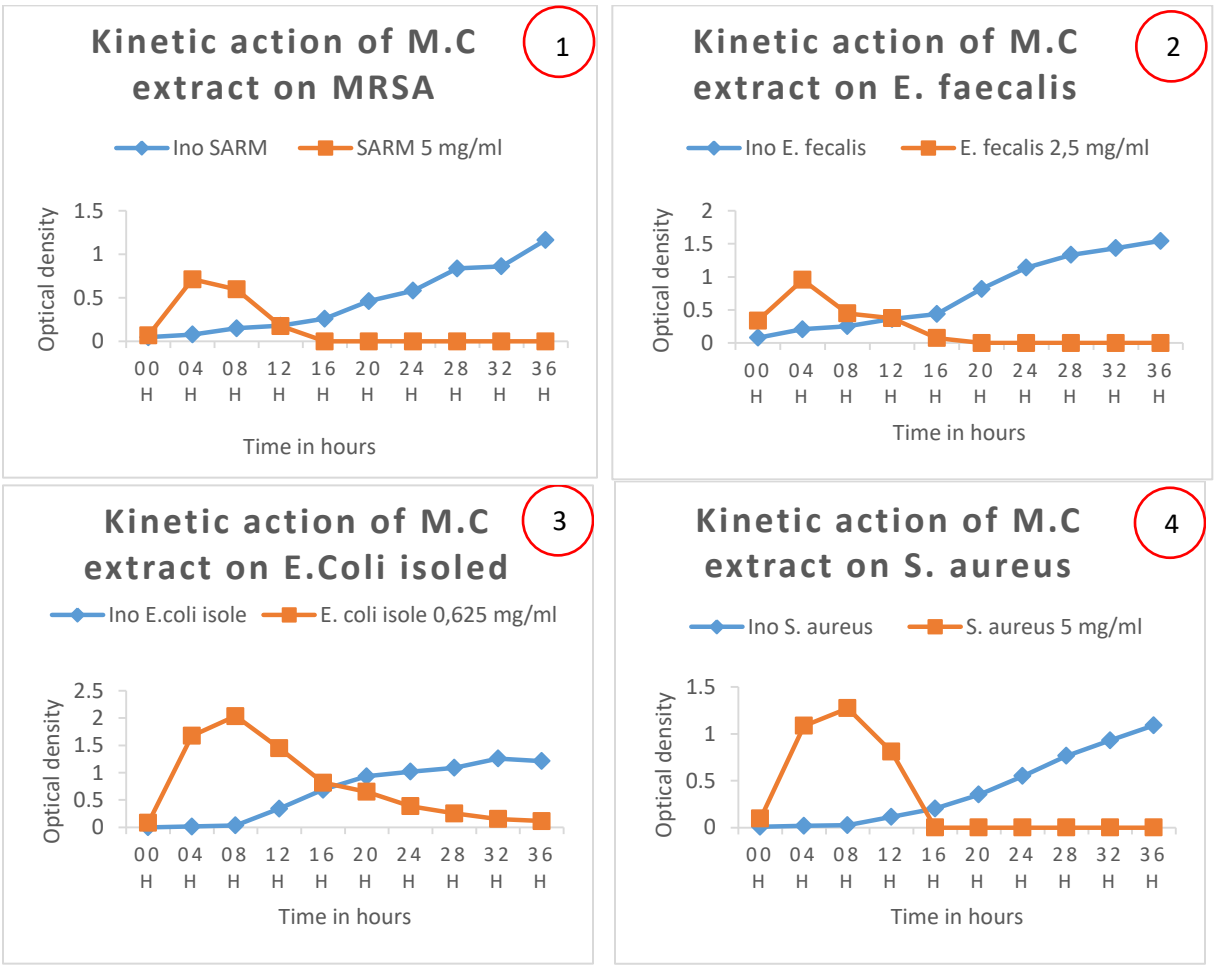

Fig 1. Action of MRSA extract - Fig 2. Action of the extract on *Enterococcus faecalis* - Fig 3. Action of the extract on *Escherichia coli* isoled - Fig 4. Action of the extract on *Staphylococcus aureus*

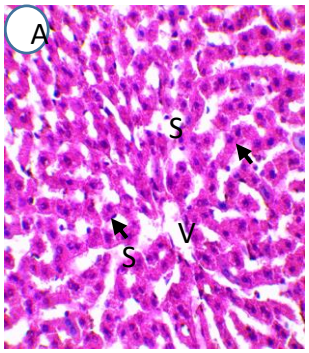

Fig 5**.** Hepatic histology of the rats of the control batch (A) and batch (B) treated with the ethanolic extract of *Momordica charantia* 5000 mg. Kg^{-1} of body weight (Gr = 400X). Centrolobular vein (V); Hepatocytes (arrows); Venous sinusoid (S).

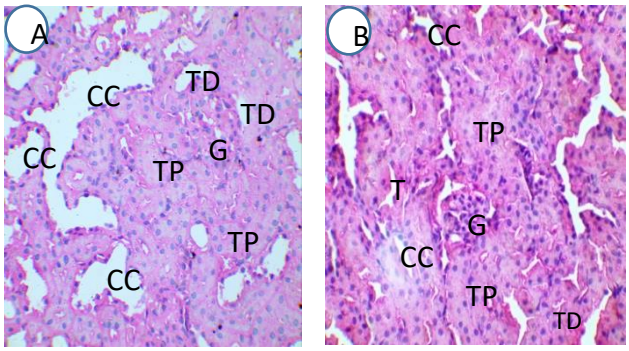

Fig 6. Renal histology of the rats of control lot (A) and batch (B) treated with ethanolic extract of *Momordica charantia* 5000 mg.Kg-1 body weight (Gr = 400X). Glomeruli (G); Proximal Tubes (PT) Distal Tubes (TD); Collector channels (CC).

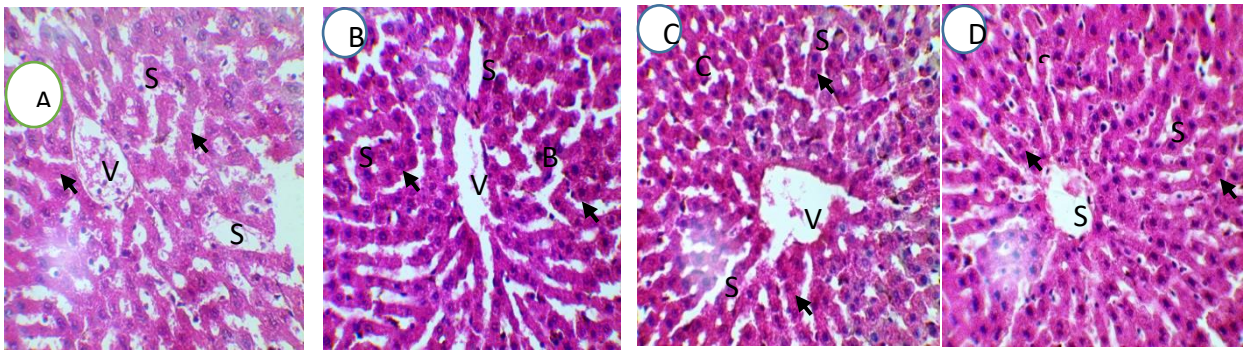

Fig 7. Hepatic histology of the rats of the control batch (A) and gaved B (500 $mg.Kg^{-1}$), C (750 $mg.Kg^{-1}$) and D (1000 $mg.Kg^{-1}$) at the same time ethanolic extract of *Momordica charantia* (Gr = 400X). Hepatocyte (arrow); Centrolobular vein (V); Venous sinusoid (S)

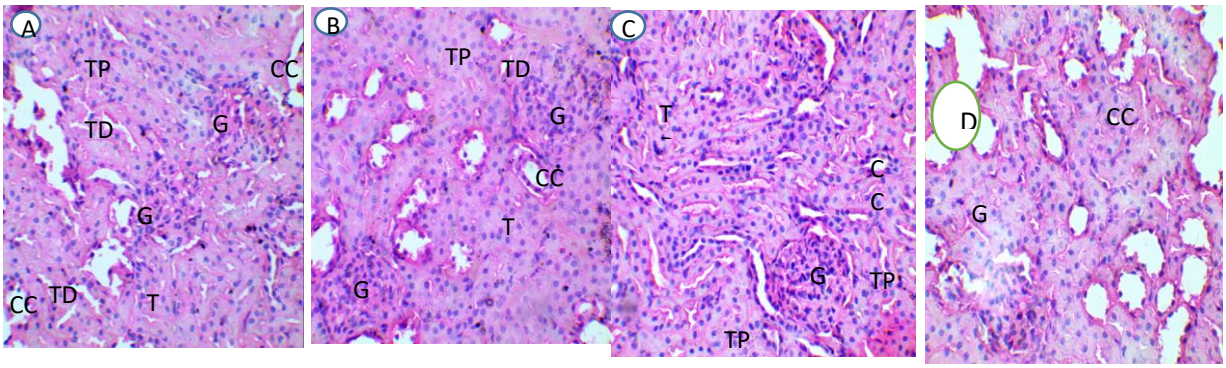

Fig 8. Renal histology rats in the control lot (A) and gaved rats, lot B (500$mg.kg^{-1}$), lot C (750 $mg.kg^{-1}$) and lot D (1000 $mg.kg^{-1}$) to the ethanolic extract of *Ocimum gratissimum* (Gr = 400X). Glomeruli (G); Proximal Tubes (PT) Distal Tubes (TD); Collector channels (CC)

These contradictions can be generated by climatic and environmental pedological factors of the places of harvest. However, all animals fed with the extract all had symptoms such as dizziness and fatigue during the first 15 to 30 minutes after force-feeding. These results are consistent with those of Husna *et al.* (2013), but contradict those of Salawu *et al*. (2004), Vikram and Sandeep (2015) and Narendra *et al.* (2016), who do not notice any behavioral changes in their animals. This behavioral variation may be due to force-feeding that would have caused stress, the

object of all these discomforts observed. The rats in the batch fed a single feed of 5000 mg / kg of weight, emitted diarrheal droppings within 24 hours after force-feeding.

The water and granule consumption of the 5000 mg / kg bw single-dose batch showed no variation from the control group (Table 8). These results corroborate those of Husna *et al.* (2013).

The gaved batch 500mg / kg bw, has not experienced any significant variation in the amount of food consumed but a significant increase in water consumption was found. As for the test batches fed daily at 750 and 1000 mg / kg bw, the pellet and water consumption decreased significantly ($p < 0.05$) (Table 9). In agreement with our results, Narendra *et al.,* (2016) found only small insignificant variations in food and water consumption.

This variation in animal nutrition is expected to affect the body weight of the animals. The body mass changes between J1 and J7 are not significant. But between J1 and J14, the mass variation is significant. ($P < 0.05$) for the batch fed to 5000 mg / kg bw (Table 10).

Regarding the control group, an increase in mass is noted but it is not significant. This means that the plant known for its high nutrient content, (Sampath *et al.,* 2010, Ullah *et al.,* 2011, Johnson *et al.,* 2016), has been able to increase the body weight of animals. These results differ from those of Abalaka *et al.* (2009) and Husna *et al.,* (2013), who found that increases in body mass were not significant after gavage with 300 and 2000 mg extracts/ kg bw.

Animals fed daily with extracts of 500, 750 and 1000 mg / kg bw, experienced a significant increase in mass after 28, 21 and 14 days respectively (Table 11). This could be explained by the existence of a adaptation before observing the effects of the extract, time, which decreases as the dose increases. These results are in contradiction with those of Narendra *et al.* (2016). These different variations have had repercussions on the biological parameters.

Hematological tests were performed to evaluate the effect of the extract on the elements of the blood cells. Hematological variations are the first indicators of toxic effects on tissues (Paprikar and Sharma, 2003). In animals, blood is the most important tissue. It is at this level that the various changes caused by the metabolic processes are noticed.

The gaved batch with an extract of 5000 mg / kg of weight showed no significant variation compared to the control, when we take the different parameters of the Blood Formula Count (Table 12). These results are in contradiction with those of Salawu *et al.* (2004) who found an increase in blood cells, and Husna *et al.,* (2013) who found a significant decrease in the number of red blood cells for animals fed with the same extract at 2000 mg / kg body weight. This could be due to the dose on the one hand and the extract on the other hand. Indeed the extract used by Salawu *et al.* (2004) is methanolic and the dose of the extract used by Husna *et al.,* Is at 2000 mg / kg of body weight.

As for the batch gaved daily with an extract of 500 mg / kg of body weight, no significant variation in the count of figured elements of the blood was observed, compared to the control batch. The batch gaved with an extract of 750 mg / kg, showed a decrease in the number of red blood cells, white blood cells and hematocrit. Platelets also decreased significantly in this lot as well as in the gaved lot at 1000 mg / kg body weight ($p < 0.05$) (Table 13). Our results are in part consistent with those of Narendra *et al.* (2016).

These data were contradictory to Husna *et al. (*2013) who found a significant increase in red blood cells and white blood cells, and a hematocrit stability in rats fed at 2000 mg / kg body weight. This could be a dose effect, since among the daily feedings, the most concentrated extract is at 1000 mg / kg of body weight whereas, according to the above-mentioned study, the extract is at 2000 mg / kg of body weight.

Among the biochemical parameters taken into account, only the urea shows a significant variation compared to the control group. Indeed, urea is a substance of hepatic origin, resulting from the metabolism of proteins. It is then eliminated by the kidney. Among the factors that may influence its increase, a high-protein diet features prominently. M. charantia is recognized as a plant containing a significant amount of protein (Krishnendu *et al.,* 2016; Johnson *et al.,* 2016). This could be the basis of this observed variation.

Our results contradict those of some authors who show that a single feeding of M. charantia fruit extract at doses up to 4000 mg / kg of extract has no significant effects on the liver (Mardani *et al.,* 2015).

The three batches tested at 500 mg / kg, 750 mg / kg and 1000 mg / kg, respectively, showed a significant increase in TGP and urea levels. Only the 500 mg / kg batch has a significant decrease in creatinine compared to the control group (Table 15). This would be due to moderate hepatic cytolysis, as GGOs did not show significant variation. According to Abalaka *et al.,* (2009), there is no significant difference in biochemical parameters between control and test animals after daily gavage at 100, 500, 800, 1200 mg / kg bw. Similarly Narendra *et al.* (2016) found no significant variation in blood parameters at the different test lots compared to the control.

After sacrifice of the rats and weight gain of the organs, no variation was observed compared to the control group (Table 16). This corresponds to the result of Narendra *et al.* (2016). On the other hand, Husna *et al.* (2013), finds after his research that organs such as liver and kidneys have significantly decreased weight compared to the

control group. Our results are also in total contradiction with those of Abalakar *et al.* (2009), for whom the liver and kidneys showed swelling, characteristic of a toxicity effect, when the animals were fed at a single dose. Kg of pc compared to the control batch.

These last observations may be due to the nature of the soil where the samples were collected. These different variations observed in the aspect of the organs, would be due either to the part of the plant used or to the quality of the environment of culture or harvest of the latter.

After the hematological and biochemical parameters, the hystopathological examinations carried out aim to help the comparison of the photographs of the cuts of the liver and the kidneys. The magnifications marked on the photographs are those used for the readings.

Liver

Macroscopic observations showed no change from the control group. Also at the microscopic level, it is noted that the liver tissues showed no difference between the treated groups and the control group.

These results are consistent with those of Tennekoon *et al.* (1994), which indicated that this plant contained a hepatotoxin able of causing cellular damage but without significant histopathological changes (Mardani *et al.,* 2015). In the study conducted by Nazrul-Hakim et al, hepatic pathology was also examined and they observed mild congestion and also found no significant differences between control and test groups at the cellular level (Nazrul - Hakim *et al.,* 2011).

Kidneys

The batch gaved at 5000 mg / kg bw showed no difference compared to the control group. These results corroborate with those of Mardani *et al.* (2015) for which gavage at 4,000 mg / kg bw showed no significant change in the kidneys. However, forced feeding for one week resulted in histopathological changes in the mouse kidneys. In the Nazrul-Hakim *et al.,* Study, histological examination showed that a dose of *Momordica charantia* extract of 1000 mg / kg for 72 hours would not result in any change in the renal structure of mice, but destruction of the tubular cells, necrosis and haemorrhage in the kidneys are observed after a gavage with fruit juice of *Momordica charantia*, for two weeks (Nazrul-Hakim *et al.,* 2011).

Also, according to Abalaka *et al.* (2009), the kidneys were more swollen when the animals were gaved at a single dose of 1200 mg / kg of bw compared to the control group. These different variations observed in the aspect of the organs, would be due either to the part of the plant used or to the quality of the environment of culture or harvest of the latter.

Metal trace elements are also the cause of toxicity in these various organs and tissues. Since *Momordica charantia*, in addition to being a medicinal plant, is popular among the food products of some nations, we used the WHO standards for food (Johnson *et al.,* 2016). The values obtained in the ETM assay are all below WHO standards. Our results are, for lead and mercury, contradicted by those of Johnson *et al*., (2016) who found that the levels of lead and cadmium in this plant are 9 times and 6.3 times higher than standard, respectively (Johnson *et al.,* 2016).

These values are lower than those obtained by Dougnon *et al.,* in 2013 in Solanum macrocarpon and from the studies made by Montcho *et al.,* (2012) in antimalarial medicinal plants, for both lead and cadmium. This variation would probably be due to the sampling sites. Indeed, the sample that was the subject of our analyzes was collected in a village far from any urbanization, while those used by Johnson *et al.* (2016) *w*ere purchased at the markets of the city of Cotonou in Benin.

Conclusion

This study has shown that *Momordica charantia* has antibacterial activity and its short use should have no harmful effect on the liver and kidney, at least not at the functional level.

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