ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Assessment of Some Serum Markers of Kidneys in Rabbits Treated By *Mitracarpus Scaber* (Rubiaceae)

Idrissa Doumbia[1]*, Karamoko Ouattara[1], Pehie Marie Thes Epse Soumahoro[1], Houphouet Felix Yapi[1,3], Allico Joseph Djaman[1,2], and Jean David N'guessan[1].

[1]Biochemical Pharmacodynamy Laboratory, Biosciences Department, Felix Houphouet-Boigny University, PO Box 582, Abidjan 22, Ivory Coast.
[2]Biochemical Laboratory of Pasteur Institute of Ivory Coast, PO Box 490, Abidjan 01, Ivory Coast.
[3]Biochemical and Molecular Biology Laboratory, Pharmaceutical and Biological Sciences Department, Felix Houphouet-Boigny University, PO Box 582, Abidjan 22, Ivory Coast.

ABSTRACT

The aim of the present study was to investigate the renal tolerance of *Mitracarpus scaber* (Rubiaceae) in rabbits. It is a plant traditionally used to treat skin diseases and various ailments in Côte d'Ivoire and elsewhere in West Africa. For this study, different batches of rabbits were injected with increasing doses of aqueous extract of *Mitracarpus scaber* (encoded Misca). Then changes in serum urea, creatinine and uric acid were evaluated. This study showed that the use of the aqueous extract of *Mitracarpus scaber* with doses between 12.5 and 200 mg / kg body weight (bw) in rabbits causes a significant variation ($P < 0.05$) of creatinine and uric acid serum concentrations. But there is no significant change ($P > 0.05$) of urea serum concentration. Finally, this study suggests that a reduction of the dose (100 mg / kg) and time of treatment (4 weeks) may help to avoid kidney dysfunction other time. This dose of 100 mg / kg/bw which is much higher than the therapeutic dose, confer on *Mitracarpus scaber* a safety margin 530 (Tolerate Maximum Dose/ Therapeutic dose) very interesting.

Keywords: *Mitracarpus scaber*, urea, creatinine, uric acid, kidney

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The climatic and environmental conditions cause the emergence of many infectious diseases in tropical countries. Among these diseases, skin diseases occupy a prominent place. In Côte d'Ivoire, as in all countries of the tropical zone, dermatitis remains a real public health problem and stands for the third reason of consultation in rural areas. Their management is made difficult by the inaccessibility to health care and the low number of dermatologists [1]. This situation obliged people to resort more and more to medicinal plants that are easily accessible [2]. This is the case of *Mitracarpus scaber* (Rubiaceae), a plant traditionally used in Côte d'Ivoire and elsewhere in Africa to treat sores, ringworm and various diseases.

The antifungal and antibacterial activities of *Mitracarpus scaber* (encoded MISCA) have been highlighted by several studies [3-7]. It has a marked activity on 12 germs among which we can quote: *Cryptococcus*, *Aspergillus, Trichophyton, Candida, Staphylococcus, E. coli,* which are opportunistic pathogens of AIDS. Minimum Fungicidal Concentration (MFC) of MISCA is 0.20 mg / ml while IC_{50} is 0.10 mg / ml [8-11]. In addition to dermatitis, it should be noted that the work of Aboughe [12] helped to highlight the cardiodepressent effect in rats.

Given the excellent results of pharmacological tests and the wide use of this plant, a rationalization of its use is required. Especially in view of its potential use in cardiovascular therapeutics. Indeed, the therapeutic use of plants is not always without danger to the user populations [13-14]. Among these risks, kidney dysfunction which represents 10.45% is one of worst in Ivory Coast [15]. Acute and subacute toxicity of MISCA in the Swiss mouse have been evaluated. The results made it possible to obtain the toxicological parameters such as the lethal dose for 50% (LD_{50}= 515 mg/kg of body weight), the lethal dose for 100% (LD_{100}=800 mg/kg bw) while the maximum tolerated dose (MTD) of the aqueous extract of MISCA is 200 mg / kg of body weight [16-17].

In the logical continuation of this work, we wanted to deepen the state of knowledge on bio-tolerance of MISCA during this study. More specifically, it is to assess the renal safety of the aqueous extract of MISCA following changes of three serum specific parameters in rabbits: urea, creatinine and uric acid. Variations in these three parameters can thus assess the impact of this extract on kidney function [18].

MATERIAL AND METHODS

Experimental

Plant material

The leaves of *Mitracarpus scaber* (Rubiaceae) collected from Abobo-Adjame Campus University (Abidjan) and in some peripheral areas of Abidjan (Côte d'Ivoire) were identified by Professor Ake Assi Laurent of Department of Botany, University of Cocody-Abidjan. A voucher specimen (N° 13612) of the plant was deposited in the herbarium of the National Floristic Center of University of Cocody-Abidjan.

Experimental animals

Rabbits, *Oryctolagus cuniculus* (36) of 8-10 weeks old, weighing 1.17±0.22 kg and bred at the Department of Biosciences, University of Cocody, Ivory Coast, were used for the experiments. They come from a rabbit cattle farm in Abidjan. The animals were kept in standard cages with good ventilation, free access to food and water. Experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences of University of Cocody-Abidjan. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals [19].

Preparation of aqueous extract of *Mitracarpus scaber* (Rubiaceae)

Plants harvested were air dried at room temperature (28±1 °C) for one month. The dried leaves were ground into fine powder. The powder (100 g) was soaked in two liters of distilled water for 48 hours on a magnetic agitator (IKAMAG RCT). The extract was filtered twice through cotton wool, and then through Whatman filter paper (3 MM). The filtrate was evaporated to dryness in a rotary evaporator (BUCHI) at 60 °C. After drying, we get a greenish powder used to prepare the aqueous extract of MISCA.

Experimental protocol

After randomization into 6 groups of 6 rabbits (3 males and 3 females), and before initiation of experiments, the rabbits were acclimatized for a period of 14 days under standard environmental conditions of temperature, relative humidity, and 12 h dark/light cycle. Animals had free access to food and water *ad libitum*.

Animals in each group were separated according to their sex in cages. Among these 6 groups, five experimental groups have received doses ranging from 12.5 to 200 mg/kg of bw (which is the Maximum Tolerated Dose (MTD) of the aqueous extract) in a geometric progression of ratio 2 [16]. Twice a week for six weeks, the animals received intraperitoneally 0.2 mL of an injection according to their group. Each rabbit of batch 1 (control) received only 0.2 mL of physiological solution of 0.09% Nacl (B. Braun) used to administrate extracts. Rabbits of batch 2 to batch 6 received respectively 12.5; 25; 50; 100 and 200 mg/kg of bw.
Blood samples were collected in the morning (from 8 to 11 h) via the marginal ear vein of the animals, once a week using sampling needles. Blood sampling was carried out once a week in the one week preceding the first application of treatment (w_0), during the five weeks of treatment (w_1, w_2, w_3, w_4, w_5 and w_6). These blood samples were collected in sterile tubes without anticoagulant. There were centrifuged at 3000 rpm for 10 min using a liquidizer JOUAN. Kidney parameters of the serum were measured with an automatic analyzer, LIASIS.

Assay for serum urea, creatinine and uric acid

The principles of the determination of each parameter are described according to the manufacturer's instructions reagents.

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Creatinine (Sprinreact): This is a method based on the Jaffe reaction. In an alkaline medium, creatinine with picric acid gives a complex of reddish orange. The speed of development of the color that is proportional to the concentration of creatinine is measured in a spectrophotometer at 500 nm wavelength.

Urea (Bio-Systems): This method is based on the Berthelot reaction. The urease hydrolyzes urea into carbon dioxide and ammonia. The ammonium ions form with salicylate, chlorine and nitroprusside a colored complex called indophenol blue-green at the concentration of urea. The intensity of the color that is proportional to the urea concentration is measured in a spectrophotometer at 600 nm wavelength.

Uric acid (Bio-Systems): In the presence of uricase, uric acid is oxidized to allantoin and hydrogen peroxide. The latter under the action of peroxidase, oxidizes dichlorohydroxybenzène sulfonate aminoantipyrine to yield a colored complex whose intensity is measured in a spectrophotometer at 510 nm wavelength.

Statistical Analysis

The data were processed using the software Graph Pad Prism 5.0 (Microsoft, USA). The analysis of variance (ANOVA) was performed according to the multiple comparison test of Tukey for the comparison of mean values of biochemical markers of different groups but also to relative baseline in each group. Data are presented means ± standard error of mean (S. E.M) for the number of animals in each group (n = 6). The difference is said to be significant if ($P<0.05$) and not significant if ($P>0.05$).

RESULTS

The results of changes in serum urea, creatinine and uric acid are expressed in tables (1, 2 and 3) are averages of six assays performed in each group.

Table 1: Effect of Misca on the levels of serum creatinine (mg/l) over time.

Serum concentrations of creatinine (mg/l)						
Doses (mg/kg)	**0**	**12.5**	**25**	**50**	**100**	**200**
w_0	10.3±3.21	5±2	10±2.64	7±1.73	11.3±1.53	8.33±1.53
w_1	11.3±1.53	4.25±1.09	11.1±2.58	7.35±0.61	12±7.21	8±1.32
w_2	8.5±3.5	5±0.5	9±1	7±2.64	10.7±1.15	6.5±0.5
w_3	10.3±1.53	7±2.64	11.3±0.58	7.67±1.26	9.67±4.51	9±1
w_4	8.33±2.89	4.17±1.25	10±5	7.7±1.54	11.7±3.04	9.2±0.98
w_5	8±1.76	7±3.46	7.67±3.78	9.8±2.03	8±0	13±1.0
w_6	11±2	7±1.73	10.3±0.0	9.67±2.52	11.7±2.89	**14±2.0***
Lots	**Lot $_1$**	**Lot $_2$**	**Lot $_3$**	**Lot $_4$**	**Lot $_5$**	**Lot $_6$**

Values are expressed as mean ± S.E.M (n = 6); * P <0.05 compared to control and to w_0 level.
w_0: Week preceding the first application of treatment; w_1 to w_6: Weeks of treatment.

Creatinine

The serum creatinine (w_0) was 10.3 ± 3.21 mg / l in the untreated lot (lot1). This value varies over time between 8 ± 1.76 mg / l (minimum w_5) and 11.3 ± 1.53 mg / l

ISSN: 0975-8585

(maximum w_1), representing a change of -22.58% (w_5) to 9.68% (w_1) of the initial rate of serum creatinine**.** In lot 2 (12.5 mg / kg), serum creatinine was 5 ± 2 mg / l before treatment. Over the past six weeks, the rate changes of 4.17 ± 1.25 mg / l (minimum w_4) to 7 ± 3.46 mg / l (maximum w_5). These values correspond to variations of -16.6% (w_4) to 40% (w_5) (Table 1).

In group 3, creatinine serum rate was 10±2.64 before treatment. This value varied to 7.67±3.78 (minimum w_5) to 11.3±0.58 (maximum w_3). These evolutions represent variations of -30.38% (w_5) to 10% (w_3).

Percentage changes as recorded in lots 4, 5 and 6 are respectively: 0% (w_2) to 40% (w_5); -29.2% (w_5) to 6.19% (w_1) and -27.78 % (w_2) to 55.56% (w_6).

Statistical analysis of the results indicates a significant change in serum creatinine ($P < 0.05$), especially with the dose of 200 mg / kg bw (lot 6) in the sixth week (Figure 1).

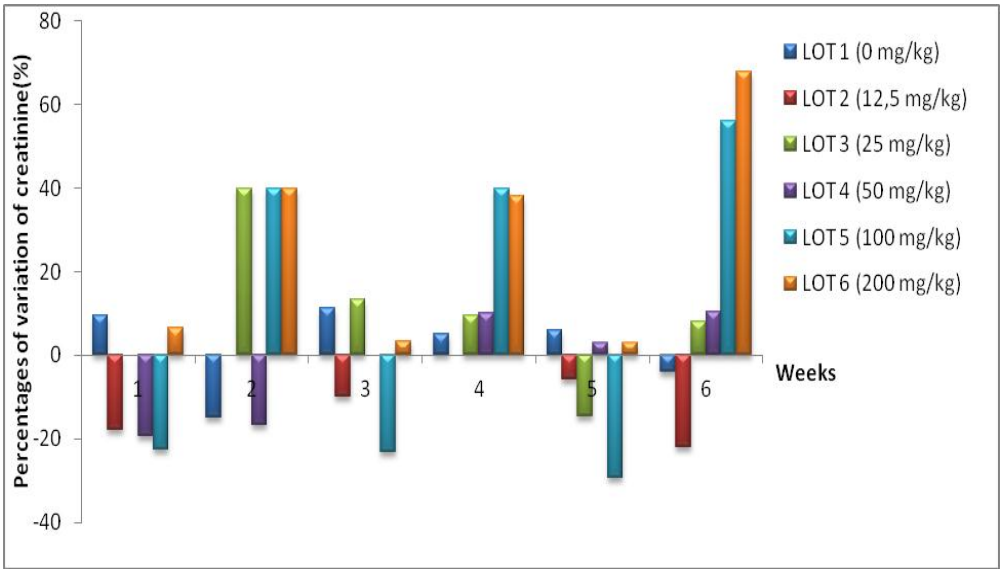

Figure 1: Percentage change of creatinine in rabbits treated and untreated by *Mitracarpus scaber* for each dose injected over time.

Urea

The serum urea (w_0) was 0.67 ± 0.16 g / l in the untreated group (group 1). This value which varies over time from 0.64 ± 0.07 g / l (minimum w_1) to 0.67 ± 0.16 g / l (maximum w_4), represents a variation of -4% (w_1) to 0% (w_4) of the initial serum urea In lot 2 (12.5 mg / kg), serum urea was 0.6 ± 0.13 g / l before treatment. Over the past six weeks, the rate changed of 0.59 ± 0.07 g /l (minimum w_6) to 0.62 ± 0.08 g / l (maximum w_4). These values correspond to variations of -1.67% (w_6) to 2.78% (w_4) (Table 2).

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Table 2: effect of Misca on the levels of serum urea (g/l) over time

Serum concentrations of urea (g/l)						
Doses (mg/ kg)	**0**	**12.5**	**25**	**50**	**100**	**200**
w_0	0.67±0.16	0.6±0.13	0.42±0.09	0.66±0.06	0.63±0.06	0.45±0.05
w_1	0.64±0.07	0.62±0.23	0.42±0.07	0.67±0.05	0.62±0.07	0.45±0.06
w_2	0.67±0.08	0.59±0.04	0.42±0.1	0.67±0.09	0.65±0.1	0.46±0.1
w_3	0.67±0.09	0.62±0.17	0.42±0.07	0.66±0.1	0.63±0.1	0.47±0.11
w_4	0.67±0.16	0.62±0.08	0.41±0.17	0.67±0.06	0.63±0.12	0.45±0.05
w_5	0.65±0.18	0.6±0.13	0.41±0.04	0.67±0.03	0.64±0.1	0.47±0.08
w_6	0.64±0.17	0.59±0.07	0.42±0.08	0.67±0.08	0.65±0.08	0.47±0.12
Lots	**Lot $_1$**	**Lot $_2$**	**Lot $_3$**	**Lot $_4$**	**Lot $_5$**	**Lot $_6$**

Values are expressed as mean ± S.E.M (n = 6); P > 0.05 compared to control and to w_0 level.
w_0: Week preceding the first application of treatment; w_1 to w_6: Weeks of treatment.

Urea serum rate in batch 3 was 0.42±0.09 during the week before treatment (**w_0**). This value changed from 0.41±0.17 (minimum w_4) to 0.42 ± 0.08 (maximum w_6). These variations represent -2.38% (w_4) to 0.79% (w_6).

Percentage changes as recorded in batches 4, 5 and 6 are respectively: -2.38% (w_4) to 0.79% (w_6); 0.50% (w_3) to 2.52% (w_4); -1.58% (w_1) to 2.63% (w_2) and - 0.73% (w_1) to 2.94% (w_3) of the initial serum urea.

The statistical analysis shows no significant change in uremia with different doses (P > 0.05) (Figure 2).

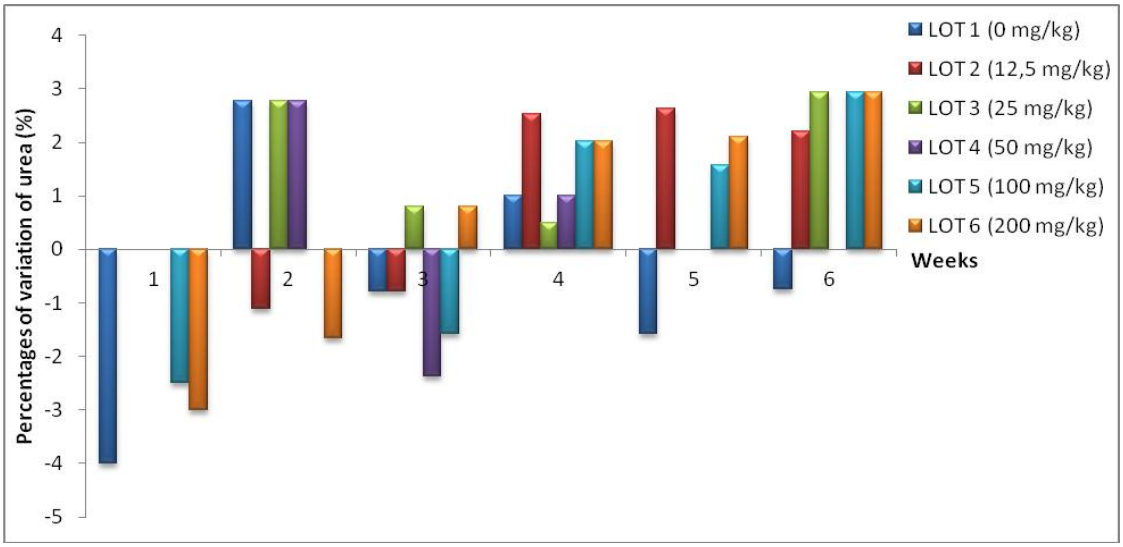

Figure 2: Percentage change of urea in rabbits treated and untreated by *Mitracarpus scaber* for each dose injected over time .

Uric acid

The serum uric acid at S_0 was 4 ± 0.58 mg / l in the untreated lot**.** This value varies over time between 4.67 ± 0.33 mg / l (minimum w_4) and 6 ± 1 mg /l (maximum w_3), a variation of 16.66% (w_4) to 50% (w_3) of the initial rate of uric acid.

ISSN: 0975-8585

In batch 2 (12.5 mg / kg), serum uric acid was 5 ± 0.57 mg / l before treatment. Over the past six weeks, the rate changed from 4.3 ± 0.3 mg / l (minimum w_1) to 6.67 ± 0.6 mg / l (maximum w_6). These values correspond to variations of -13.33% (w_1) to 33.33% (w_6) of the initial serum uric acid (Table 3).

Table 3: Effect of Misca on the levels (mg/l) of serum uric acid over time.

Serum concentrations of uric acid (mg/l)						
Doses (mg/kg)	**0**	**12.5**	**25**	**50**	**100**	**200**
w_0	4±0.58	5±0.57	5.67±0.3	7±0.5	6.33±0.3	6±0.58
w_1	5±0.58	4.3±0.3	5±1	5.67±1.2	7±1.52	5.67±1.33
w_2	5.3±1.86	4.3±0.88	5.33±0.6	6.67±1.4	5±0.58	6.67±0.8
w_3	6±1	5.67±1.2	6±1.15	7.33±1.2	6.33±0.8	7±0.58
w_4	4.67±0.33	4.67±0.88	5±1	7.33±0.8	7±0.58	8.67±0.88
w_5	5±1	6±1	6±0.58	5.33±0.8	7.33±0.3	**9.67±0.88***
w_6	5±0.58	6.67±0.6	6.33±0.3	7±0.57	7.33±1.2	**10.3±0.58****
Lots	**Lot $_1$**	**Lot $_2$**	**Lot $_3$**	**Lot $_4$**	**Lot $_5$**	**Lot $_6$**

Values are expressed as mean ± S.E.M (n = 6); * P < 0.05; **P < 0, 01 compared to control and to w_0 level.
w_0: Week preceding the first application of treatment; w_1 to w_6: Weeks of treatment.

Before treatment, uric acid serum rate was 5.67±0.3 in lot 3. This value varied from 5±1 (minimum w_1, w_4) to 6.33±0.3 (maximum w_6). These variations correspond to 11.76% (w_1) to 11.76% (w_6). In group 4, uric acid serum rate was 7±0.5 during w_0. The percentage change during the weeks of treatment is -23.8% (w_5) to 4.76% (w_3).

The percentage changes so recorded in batches 5 and 6 are respectively -21.05% (w_2) to 15.79% (w_6) and -5.55% (w_1) to 66.66% (w_6).

Statistical analysis of the results indicates a significant change in serum uric acid (P <0.05), especially with the dose of 200 mg / kg bw (lot 6) in the fifth and sixth week (Figure 3).

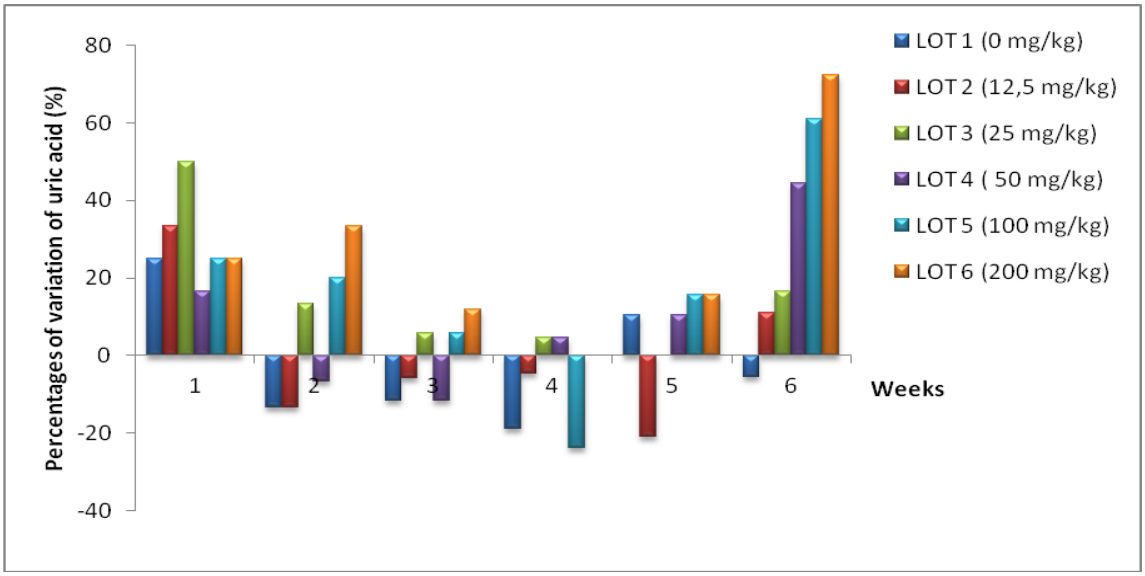

Figure 3: Percentage change of uric acid in rabbits treated and untreated by *Mitracarpus scaber* for each dose injected over time.

ISSN: 0975-8585

DISCUSSION

Variations in serum activities of enzymes stored in different batches before treatment and those recorded in the control group (batch 1) which has not undergone any treatment are in conformity with the usual values obtained in rabbits [20].

Statistical analysis of the results indicate that the aqueous extract of MISCA with the doses between 0 and 200 mg / kg body weight for six weeks, don't lead a significant change in blood urea. But there is a significant change in serum creatinine and uric acid. These variations are more pronounced with the dose of 200 mg / kg body weight especially during the fifth and sixth week.

Indeed, creatinine, urea as uric acid are substantially removed from the blood by glomerular filtration. It means that, the concentrations of these metabolites in urine are regulated by the kidney which has a real role of blood filter. It is also established that the glomerular filtration rate is dependent on the pressure in the glomerular capillaries about 30 mm Hg. The decline in blood pressure can cause a decrease in glomerular pressure about 10 mmHg. Any decrease in blood pressure may decrease plasma volume filtered by the kidney. Thus, metabolite concentrations which are not correctly eliminated increase in the blood. That is here the case of creatinine and uric acid. This is one of the leading causes of kidney failure [21,22].

In fact, the link between changes in blood pressure and the occurrence of renal failure have been revealed by many authors [23-24]. This phenomenon has been described with other plants such as *Phyllanthus amarus* (Euphorbiaceae) and *Mareya micrantha* (Euphorbiaceae) [25, 26]. This could therefore suggest an induction of renal dysfunction with very high doses of the aqueous extract of MISCA. Indeed, MISCA would have a cardiodepressant activity on isolated rat heart coupled with an hypotensive effect on blood pressure at 4.5 mg / L. The mechanism of this hypotension is considered to be the same as calcium antagonists because it is suppressed by a calcium provide[12].

In addition, the metabolism of several well-known calcium antagonists such as nifedipine and verapamil indicate that the kidney plays an important role in eliminating them. For example, 70-80 % of nifedipine is excreted by the kidneys, more than 90% of this amount is recovered in the urine after 24 hours, while the metabolites of verapamil, are excreted exclusively via the kidney for 70% [27-30]. These data confirm to wish that the kidney may play a key role in the elimination of the aqueous extract of *Mitracarpus scaber* like that of some calcium antagonists.

CONCLUSION

At the end of this work, it appears that the use of aqueous extract of *Mitracarpus scaber* at doses between 12.5 and 200 mg / kg of body weight in rabbits causes a non significant change in serum urea and a significant change in serum creatinine and uric acid. This increase is noticeable during the two last weeks with the dose of 200 m / kg bw. The high doses of *Mitracarpus scaber* (more than 100 mg / kg bw) could lead renal dysfunction. This study suggests that a reduction of the dose (100 mg / kg bw) and time of treatment (4

weeks) may help to avoid kidney insufficiencies in the long term. We note that with this dose of 100 mg / kg (106 mg / ml) which is much higher than the therapeutic dose (CMF= 0,2 mg/ml), *Mitracarpus scaber* always keep a safety margin of 530 very interesting.

However, it is necessary that the traditional use of this plant in decoction to relieve various ailments must be rationalized. Moreover, in order to better understand all aspects of bio-tolerance, it would be necessary to carry out further studies including cardiovascular and liver tolerance as well as, urinary metabolites, hematological and histological investigations.

ACKNOWLEDGMENTS

The authors are grateful to Pr Ake Assi of the Department of Botany and Pr Djessou Prosper of Medical Biochemistry Department, University of Cocody-Abidjan for their respective substantial contributions in botanical identification and collection of the plant and dosages of serum markers.

REFERENCES

[1] Caumes E. Atlas de dermatologie tropicale (Tome I). Janssen Cilag Laboratories, 1998, 88 p.
[2] Pousset JL. Medicinal Plants of Africa. How to recognize and use? La Calade, 2004, 287 p.
[3] Crockett CO, Guede-Guina F, Pugh DA, Vangah MM, Bodo B, Smith MO, Ochillo RF. The FASEB J 1992; 6(4):1590.
[4] Guede-Guina F, Vangah MM, Harouna D, Bahi C. J Ethnopharmacol 1993;25(5):23-29.
[5] Bisignano G, Sanogo R, Marino A. Lett Appl Microbio 2000;30:105-108.
[6] Gbaguidi F, Accrombessi G, Gbenou J, MoudachirouM., Quertin-Leclercq J. J Chem Soc West Africa 2000;22:13-20.
[7] Koffi A, Dally I, Aka Coulibaly S, Guede-Guina F. J Sci Pharm Biol 2007;8(2):18-24.
[8] Guede-Guina F, Vangah MM, Harouna D, Bahi C. Rev Med Pharm 1995;9(1):13-19.
[9] Guede-Guina F, Kra AM, Vangah MM, Bonga GM. ABM 1997;2(1):11-16
[10] Mobie DP, Bonga GM, Vangah MM, De Souza C, Guede-Guina F. Rev Med Pharm 1997;3:15-19.
[11] Okou OC, Zirihi GN, Kra AM, Dosso M, Guede-Guina F. J Sci Pharm Biol 2006;1:17-27.
[12] Aboughe AS**.** Evaluation of cardiodepressant effect of Misca compared with actions of common calcium antagonists, DEA, Biotech. Pharm, Biosciences, Univ. Cocody, Abidjan, 29 p.
[13] Auzephy PH and Manigand G. Drugs accidents. Ellipses, 1990, 446 p.
[14] Bruneton J. Toxic plants. Plants dangerous to humans and animals. Paris, 2nd Edition Tec and Doc (International medicine), 2001,
[15] Gnionsahe DA, Coffi DA , Mignonsin D, and Yapobi Y. Nephr 1995;3(3):270-305.
[16] Doumbia I, Djyh BN, Adebo IB, Guede-Guina F, Djaman AJ. Rev Ivoir Sci Technol 2006;8:207-215.
[17] Doumbia I. Toxicité et bio-tolerance de *Mitracarpus scaber* et *Mareya micrantha* chez la souris et le lapin. Doctorat, Université de Cocody-Abidjan, 2008; 208 p.
[18] Dieusaert P. Practical guide to laboratory tests. Maloine, 2005; 1543 p.

[19] Anonymous. Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes Official Journal L 358, 18/12/1986; P. 0001-0028.
[20] Coulibaly FA, Coulibaly A, N'guessan JD, Guede-Guina F. Sci Nat 2006; 4: 37-43.
[21] Ichikawa I, Maddox DA, Cogan MG, Brenner BM. Renal Physiol 1978; 1: 121-131.
[22] Djyh BN. Etude de l'évolution de quelques parameters sériques marqueurs de la bioltolérance de trios phytomédicaments. Thèse de Doctorat 3è cycle, UFR, Biosciences, Université, Cocody-Abidjan ; 2003,144p.
[23] Lindeman RD, Tobin JD, Shock NW. Kidney Int 1984;26:861-864.
[24] Klag MJ, Whelton PK, Randall BI. N Engl J Med 1996; 334: 13-18.
[25] Coulibaly FA, Djyh BN, Guede-Guina F. Djaman AJ. Ann Bot Afr O 2007; 5: 69-78.
[26] Doumbia I, Adebo IB, Coulibaly FA, Djaman AJ, Guede-Guina F. J Sci Pharm Biol 2007; 8(2):41-49.
[27] Epstein M. J Cardiovasc Parmacol 1994;24(Suppl.A)S: 18-24.
[28] Bidani AK and Griffin KA. J Lab Clin Med 1995; 125: 553-555.
[29] Vidal. The dictionary, 2013.