ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Renal Tolerance Study of the Ethyl Acetate Fraction of *Morinda morindoides* (Baker) Milne-Redhead (Rubiaceae) Extract in Rabbit

IRIE OTIS TRA-BI[1*], BERNARD NAZAIRE DJYH [1], DODEHE YEO[1], MARIE PASCALE ITO Mrs TEBELE[1], ALLICO JOSEPH DJAMAN [1,2], JEAN DAVID N'GUESSAN [1]

[1]Biochemical Pharmacodynamy Laboratory, Biosciences Department, Cocody University PO Box 582, Abidjan 22, Ivory Coast
[2]Pasteur Institute of Ivory Coast, PO Box 2393, Abidjan 04, Ivory Coast

ABSTRACT

The effect of the ethyl acetate fraction, from the hydro-alcohol extract of *Morinda morindoides* (Baker) Milne-Redhead (Rubiaceae), was evaluated on the tissue and biochemical markers of kidney in Rabbits. Five groups of 6 rabbits each, were injected intraperitoneally, twice a week for four weeks, with this fraction , dosed from 25 to 100 µg/kg body weight, for Groups 2 to 5 while Group 1, received 1 ml of Mac Ewen physiological fluid. Blood sampling carried out to evaluate urea, creatinine, uric acid, sodium and potassium changes, showed only slight changes of uric acid and potassium concentrations ($p< 0.05$) but these changes were within the limits of their normal values in rabbit. Histopathological studies conducted, the sixth week, on the kidneys of two rabbits of each group revealed undamaged tissues. These results revealed that the ethyl acetate fraction of *Morinda morindoides*, used in this study, may be well tolerated by the kidney.

Keywords: Biochemical markers, histopathology, *Morinda morindoides*, renal tolerance.

Corresponding author

INTRODUCTION

The use of plant-based systems continues to play an essential role in health care. It has been estimated that approximately 80 % of population in the developing countries depend on traditional medicine for their primary health care [1, 2]. Because of this strong dependence on plants as medicine, ethnopharmacological studies have been conducted to determine their safety and efficiency and on the other hand to find out new active principles from plants [3].

Morinda morindoides is one the plants used in traditional medicine practice in Nigeria, Democratic Republic of Congo, in Congo-Brazzaville where its leaves are frequently used against malaria, diarrhoea, amoebiasis, hemorrhoids, gonorrhea and rheumatic pains [4-8].

In Cote d'Ivoire, the leaf of this plant is traditionally used by population in the West central against diarrhea [9, 10]. Another studies to support the traditional use of this plant showed antifungal and antibacterial activities [11, 12].

Studies performed to improve these antidiarrheal, antifungal and antibacterial activities, have allowed to isolate different extracts and fractions; from this plant. Among the different fractions tested, the ethyl acetate fraction F1 of the hydroalcohol extract, composed of alkaloids and sterols, offered the most promising biological activity [11, 13].

To guarantee the safety use of *Morinda morindoides* by populations, early study of the hepatic tolerance of this fraction revealed that it is well tolerated by liver [14]. But up now, there are no available scientific data concerning the influence of this fraction on the kidney.

Whereas, the kidney is the most highly innervated peripheral organ, and both the excretory and endocrine functions of the kidney are regulated by renal nerve activity. The kidney plays a dominant role in body homeostasis, blood ionic concentration and pH; thereby it contributes importantly to systemic blood pressure control [15]. In the other hand, the high renal blood flow and process of concentration of drugs and their metabolites during formation of urine, predisposes kidneys to toxic drug injury [16].

This study was therefore conducted, referring to the hepatic tolerance study's conditions, to assess the effect of the ethyl acetate fraction (F1) of *Morinda morindoides* on some biochemical markers and tissue of the kidney.

MATERIALS AND METHODS

Experimental

Plant material

The leaves of *Morinda morindoides* (Baker) *Milne-Redhead* (Rubiaceae) were collected in the region of Daloa, west-central Ivory Coast.

The plant was authenticated by Professor Ake Assi of the Department of Botany, University of Cocody-Abidjan and a voucher specimen (no. 17710) of the plant was deposited in the herbarium of the National Floristic Center of University of Cocody- Abidjan.

Preparation of *Morinda morindoides* extract

The leaves of *Morinda morindoides* (Baker) *Milne-Redhead* (Rubiaceae) were air-dried at a room temperature (28 ±1 °C) for 7 days and ground into fine powder. The powder was mixed with distilled water (80 g in 2 L of distilled water) for 24 h with constant stirring at 80 °C. The extract was filtered twice through cotton wool, and then through Whatman filter paper no. 1. The filtrate was evaporated to dryness in a rotary evaporator (Buchi) at 60 °C. Twenty five grams of the dry aqueous extract was added to 500 ml of ethanol and water (consisting of 356 ml of ethanol 96 % and 144 ml of distilled water) and after thorough mixing, the supernatant was evaporated in a rotary evaporated following the method of Guede-Guina et al [17]. The residue was taken as the hydro-alcohol,extract.

Preparation of the chromatographic fraction of the extract

Five grams (5 g) of the hydro-alcohol extract was added to 500 ml of water/ethyl acetate mixture (consisting of 250 ml of distilled water and 250 ml of ethyl acetate) and stirred continuously for 24 h. After decantation, the supernatant was evaporated in a rotary evaporator. The residue was taken as the ethyl acetate extract of the plant leaf (AcE). The ethyl acetate extract was subjected to separation on a chromatographic column (2 x 50 cm) with silica gel 60 (Merck, silica gel, 0.063 – 0.200 mm). The extract (0.25 g) was introduced into the column and placed on the silica gel, protected by cotton. Dichloromethane was used as eluent until total infiltration of the extract into the silica gel; thereafter, dichloromethane-methanol (95/5) mixture was used for elution until separation was completed.

The four fractions obtained, based on their colour, were F1 (golden yellow), F2 (dark green), F3 (pale green) and F4 (yellow orange).[11] Fraction F1 (AcE F1) was evaporated in a rotary evaporator (at 60°C) before used in the *in vivo* studies.

Experimental animals

Rabbits, *Oryctolagus cuniculus*, from a rabbit cattle farm in Bingerville, southern Ivory Coast were used in this study. The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, University of Cocody-Abidjan. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals [18].

Administration of fraction F1

Thirty (30) rabbits (15 males and females each) were acclimatized for a month and half at ambient temperature (28±1 °C) and humidity (70 ± 5%). Their age was approximatively 3 months and weighed 1.2 ± 0.2 kg on the average. They were randomly divided into 5 groups of 6 rabbits each (3 males and 3 females). Animals in each group were separated according to their sex in cages (length: 79 cm, width: 50 cm and height: 38 cm). They had free access to both water and food.

Twice a week for four weeks, the animals received intraperitoneally (ip) 1 ml of an injection according to their group. Group 1 (Gp1), control, received 1ml of Mac Ewen physiological fluid while Groups 2 (Gp2) to 5 (Gp5) were similarly injected with 30, 60, 60 µg/ml, 90 and 120 µg/ml, respectively, of AcE F1, equivalent to 25, 50, 75 and 100 µg/kg body weight, respectively.

Collection of blood

Blood samples were collected in the morning (from 8 to 11 am) via the marginal ear vein of the animals. Blood sampling was carried out once a week in the two weeks preceding the first application of treatment (S0), during the four weeks of treatment (S1, S2, S3 and S4), and then for two weeks after the treatment period (S5 and S6). These blood samples were collected in tubes (without anticoagulant) and centrifuged at 3000 rpm for 10 min. The serum was stored at -20 °C until analyzed for enzymatic activities and concentration of biochemical metabolites.

Assay of renal parameters in rabbit serum

Table 1: Operating parameters for the quantitative determination of metabolites and electrolytes.

Parameter	Colorimetric Method	Wavelength (nm)
Creatinine	Alkaline picrate	500
Urea	Urease	600
Uric acid	Uricase, peroxidase	510
Sodium	Temperature (2000°K)	589
Potassium	Temperature (2000°K)	767

In exception to sodium and potassium whose were measured by flame photometer, SEAC *fp* 20, renal parameters of the serum were measured with an automatic analyzer, Hitachi 902 (Roche), using commercial kits and certified controls (Spinreact S.A., Ctra Santa Coloma, Spain) based on the manufacturer’s instructions, as summarized in Table 1.

Preparation of tissue sections and histopathology

After the blood collection the last week (S6), two rabbits of each group were randomly chosen and sacrificed under deep anesthesia and kidneys were removed and fixed with 10% buffered formalin for further analysis.

Renal tissues were cut into transverse blocks. An automatic processor (RH-12EP Sakura, Fine Technical Co.Ltd, Tokyo, Japan) was used for further processing blocks. About 12 hours were required for dehydration (96% alcohol for one hour x six changes, and 100% alcohol for one hour x one change).

Clearing in three changes of xylene for one hour each. Tissues were impregnated in two changes of paraffin wax with a melting point of 50° C for period of 2 hours. Embedding of tissue was done in paraffin using L-shaped metallic moulds. These blocks were put in the refrigerator for a period of 4-6 hours. Each block was cut on a rotary microtome (Microm Gmbh, Waldorf, Germany). About 4-5 micro meter thick tissue section were obtained and placed in the water bath with temperature of 50°C below the melting point of paraffin wax. Cut ribbons of tissues were placed on the albumenized glass slide.

All the sections and smears (both touched and scraped) were stained with haematoxylin and eosin (H.E). These sections were examined photo microscopically for renal damage.

Statistical analysis

The results are presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) with repeated measures was employed to compare the results according to the administered doses and times of treatment. Analysis of variance was considered significant when the level of probability *(p)* was < 0.05. When the value of *p* was significant, a Post-Hoc test of Newman-Keuls was carried out. These analyses were carried out with the software, Statistica 7.1 (Statistica, Statsoft) using a general linear model (GLM).

RESULT

Serum parameter

Tables 2 to 7 show the effect of the ethyl acetate fraction F1 of the hydro-alcohol extract of *Morinda morindoides* (AcEF1) on serum metabolites and serum electrolytes. Levels of urea (p=0.9688), creatinine (p= 0.7250) and sodium (p=0.0827) were statistically unchanged but those of uric acid (p=0.0160) and potassium (p=0.0001) changed during the present study with highest level of potassium in group 5 at S4 and lowest value of uric acid in group 3 at S5. In the other hand, the effect of AcEF1 on these parameters was not dose dependent.

ISSN: 0975-8585

Table 2: Effect of AcEF1 on the levels (g/L) of serum urea

Group	Dose (µg/Kg)	S0	S1	S2	S3	S4	S5	S6
Gp1	0	0.18 ± 0.03	0.19 ± 0.02	0.19 ± 0.03	0.18 ± 0.02	0.19 ± 0.02	0.18 ± 0.01	0.19 ± 0.02
Gp2	25	0.20 ± 0.03	0.21 ± 0.04	0.19 ± 0.03	0.18 ± 0.02	0.19 ± 0.02	0.18 ± 0.01	0,18 ± 0.04
Gp3	50	0.20 ± 0.02	0.20 ± 0.03	0.19 ± 0.03	0.19 ± 0.03	0.18 ± 0.02	0.19 ± 0.01	0.18 ± 0.03
Gp4	75	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.19 ± 0.02	0.19 ± 0.01	0.19 ± 0,02	0.20 ± 0.02
Gp5	100	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.03	0.19 ± 0.02	0.20 ± 0.02	0.20 ± 0.02	0.19 ± 0.02

Values are expressed as mean ± S.E.M (n = 6). Values without common superscript letter (a-b)* in column and row differ ($p < 0.05$). *a<b. S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period.

Table 3: Effect of AcEF1 on the levels (mg/L) of serum uric acid

Group	Dose (µg/Kg)	S0	S1	S2	S3	S4	S5	S6
Gp1	0	4.77^{b} *± 0.78*	4.78^{b} *± 0.66*	4.87^{b} *± 0.25*	4.73^{b} *± 0.48*	4.87^{b} *± 0.65*	4.76^{b} *± 0.54*	4.35^{b} *± 0.44*
Gp2	25	4.69^{b} *± 0.37*	4.68^{b} *± 0.57*	4.42^{b} *± 0.60*	4.43^{b} *± 0.74*	4.92^{b} *± 0.46*	4.90^{b} *± 0.82*	5.08^{b} *± 0.73*
Gp3	50	4.85^{b} *± 0.39*	4.58^{b} *± 0.73*	4.57^{b} *± 0.74*	5.13^{b} *± 0.47*	4.83^{b} *± 0.91*	3.91^{a} *± 0.54*	5.03^{b} *± 0.39*
Gp4	75	4.06^{b} *± 0.52*	4.40^{b} *± 0.46*	4.50^{b} *± 0.53*	5.22^{b} *± 0.50*	5.13^{b} *± 0.63*	4.59^{b} *± 0.51*	5.12^{b} *± 0.78*
Gp5	100	4.64^{b} *± 0.73*	5.27^{b} *± 0.58*	4.63^{b} *± 0.68*	4.62^{b} *± 0.59*	4.55^{b} *± 0.74*	4.75^{b} *± 0.55*	4.70^{b} *± 0.30*

Values are expressed as mean ± S.E.M (n = 6). Values without common superscript letter (a-b)*in column and row differ ($p < 0.05$). *a<b. S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period

Table 4: Effect of AcEF1 on the levels (mg/L) of serum creatinine

Group	Dose (µg/Kg)	S0	S1	S2	S3	S4	S5	S6
Gp1	0	7.50 *± 1.09*	8.17 *± 1.47*	8.50 *± 1.87*	7.33 *± 1.37*	7.17 *± 1.17*	7.50 *± 1.38*	6.83 *± 1.47*
Gp2	25	7.44 *± 0.58*	8.17 *± 1.17*	7.17 *± 1.47*	7.83 *± 1.17*	7.83 *± 1.17*	7.50 *± 1.05*	7.50 *± 1.05*
Gp3	50	7.39 *± 0.44*	7.50 *± 1.05*	7.83 *± 1.47*	8.67 *± 1.51*	6.83 *± 1.47*	7.50 *± 1.05*	7.50 *± 1.05*
Gp4	75	7.89 *± 0.46*	7.50 *± 1.17*	8.33 *± 1.51*	7.00 *± 1.10*	7.67 *± 1.37*	7.50 *± 1.05*	7.50 *± 1.05*
Gp5	100	7.61 *± 1.04*	8.17 *± 1.47*	8.50 *± 1.05*	7.33 *± 1.51*	7.33 *± 1.51*	8.00 *± 0.89*	6.83 *± 1.17*

ISSN: 0975-8585

Values are expressed as mean ± S.E.M (n = 6). Values without common superscript letter (a-b)* in column and row differ (p < 0.05). *a<b. S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period.

Table 5: Effect of AcEF1 on the levels (mEq/L) of serum sodium

Group	Dose (µg/Kg)	S0	S1	S2	S3	S4	S5	S6
Gp1	0	139.39 ± 4.28	139.83 ± 3.82	143.67 ± 4.72	140.50 ± 1.76	140.00 ± 2.53	139.67 ± 1.21	141.33 ± 1.86
Gp2	25	139.61 ± 1.73	140.67 ± 3.72	144.17 ± 3.71	140.33 ± 2.50	140.50 ± 1.38	142.83 ± 4.71	140.83 ± 2.48
Gp3	50	139.39 ± 2.42	143.83 ± 3.71	141.67 ± 3.88	140.83 ± 2.14	140.50 ± 1.64	140.83 ± 2.04	141.83 ± 2.40
Gp4	75	141.72 ± 1.58	140.17 ± 2.04	142.33 ± 1.63	142.00 ± 1.55	140.33 ± 1.37	140.17 ± 2.14	141.00 ± 1.67
Gp5	100	136.28 ± 4.01	139.50 ± 2.43	141.67 ± 3.20	142.50 ± 3.45	142.67 ± 2.66	141.33 ± 2.88	140.67 ± 1.63

Values are expressed as mean ± S.E.M (n = 6). Values without common superscript letter (a-b)*in column and row differ (p < 0.05). *a<b. S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period.

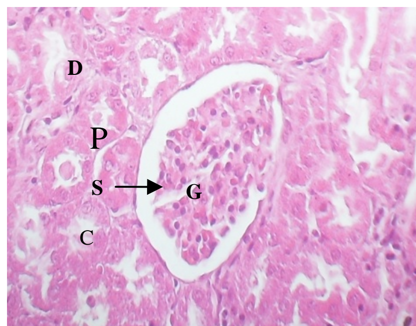
Table 6: Effect of AcEF1 on the levels (mEq/L) of serum potassium

Group	Dose (µg/Kg)	S0	S1	S2	S3	S4	S5	S6
Gp1	0	4.06^b ± 0.25	4.00^b ± 0.18	4.20^b ± 0.28	4.12^b ± 0.41	4.08^b ± 0.29	4.10^b ± 0.28	4.00^b ± 0.09
Gp2	25	3.92^b ± 0.07	4.23^b ± 0.39	4.23^b ± 0.27	4.02^b ± 0.36	4.05^b ± 0.15	4.03^b ± 0.12	4.02^b ± 0.10
Gp3	50	3.85^b ± 0.26	4.17^b ± 0.39	3.78^b ± 0.40	4.27^b ± 0.42	4.23^b ± 0.45	4.17^b ± 0.36	4.03^b ± 0.10
Gp4	75	3.71^b ± 0.28	4.18^b ± 0.34	4.08^b ± 0.16	3.90^b ± 0.19	4.15^b ± 0.46	4.18^b ± 0.34	4.08^b ± 0.15
Gp5	100	3.38^a ± 0.31	4.02^b ± 0.34	4.08^b ± 0.27	4.10^b ± 0.17	4.52^c ± 0.45	4.18^b ± 0.34	4.03^b ± 0.15

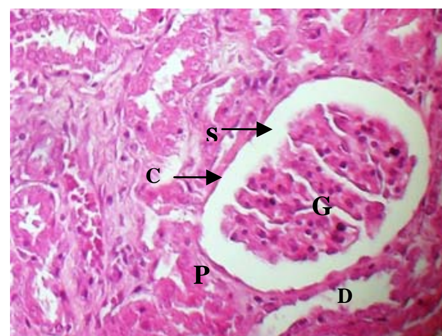
Values are expressed as mean ± S.E.M (n = 6). Values without common superscript letter (a-b)* in column and row differ (p < 0.05). *a<b. S0=Two weeks preceding the first application of treatment; S1 to S4 =Weeks of treatment; S5 and S6 = Weeks after the treatment period.

Histopathological study

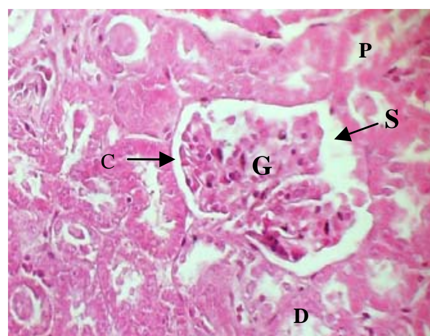
Figures 1-a to 1-e present photomicrograph of renal cortex in control group (Group 1) and experimental groups (2-5). Compared to the control group, experimental groups have presented normal renal cortex structure without damage with Bowman's capsule, Bowman's space, glomerulus, proximal convoluted tubule (with brush border or microvilli) and distal convoluted tubule (absent or few microvilli) were clearly observed.



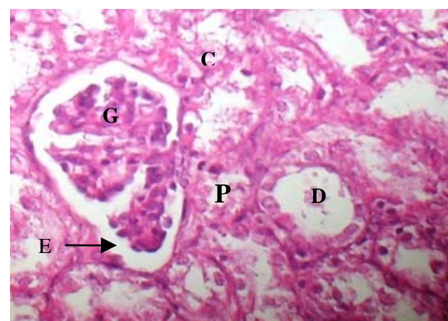
a-Group 1



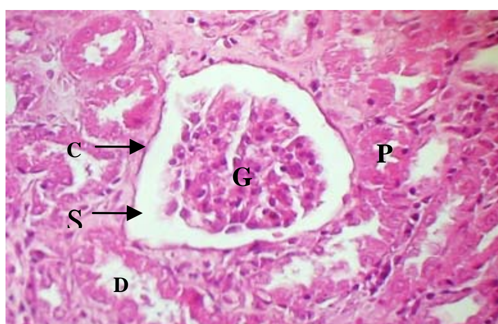
b-Group 2



c-Group 3



d-Group 4



e-Group 5

DISCUSSION

In the present study, the values of creatinine, urea, uric acid, sodium and potassium found for the control group are in conformity with their respective normal values reported by Founzegue et al. [19].

Among these parameters tested, only uric acid and potassium levels changed slightly but changes observed were not dose dependent and, furthermore, they were within the limits of their standard values in rabbit.

Defined by India's National Institute of Health (NIH) 2001, a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological, pathologic processes, or pharmacologic responses to a therapeutic intervention [20].

Disruption in renal function is revealed by the changes of theses biomarkers because of the intervention of the kidney in the blood concentration of each biomarker through different mechanisms:

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass [21]. Creatinine is a commonly used as measure of kidney function. The diagnosis of renal failure is usually suspected when serum creatinine is greater than the upper limit of the "normal" interval. Serum creatinine concentration increase in the presence of impaired renal function [22].

Urea is major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. In kidneys urea is filtered out of blood by glomerulli and is partially being reabsorbed with water [23]. The most frequently determined clinical indices for estimating renal function depends upon concentration of urea in the serum [22].

Uric acid is the end product of purine metabolism. The major rate limiting step in the synthesis of uric acid is the intracellular concentration of 5-phosphoribosyl 1- pyrophosphate (PRPP). Uric acid serves no Biologic function. Approximately two third of uric acid is excreted by the kidney and one third through the gastrointestinal tract. Of the total uric acid filtered through the glomerulus, 98-100 % of this glomerular filtrate is reabsorbed in the proximal portion of proximal convulated tubule, eventually 6 to 12 % of the original glomerular filtrate is excreted [22].

Potassium used as a most convincing electrolyte marker of renal failure. The combination of decreased filtration and decreased secretion of potassium in distal tubule during renal failure cause increased plasma potassium. Hyperkalemia is the most significant and life-threatening complication of renal failure [24].

Sodium content of the blood is a result of a balance between the amount in the food and beverages consumed, and the amount of kidneys excretion. In addition, a small percent is lost through the stool and sweat.) Many factors affect sodium levels, including the steroid hormone aldosterone, which decreases loss of sodium in the urine [25].

These findings of the present study showed that the use of the ethyl acetate fraction F1 of the hydro-alcohol extract of *Morinda morindoides* (AcEF1), in the tested dose range, would not disrupt the main functions of the kidney: glomerular filtration, tubular secretion and tubular excretion.

The keeping of glomerular filtration and tubular functions (reabsorption, secretion and excretion) would be explained by non-effect of AcEF1 not only on mechanisms which intervene in these functions (revealed by the changes of renal markers) but also on structures of different parts of the nephron, responsible of these functions.

Histopatological studies revealed undamaged structures of the kidney (glomerulus, proximal convoluted tubule and distal convoluted tubule).

These results showed that the AcEF1 would not disrupt the main functions and the integrity of kidney. These results are in agreement with previous studies where it was reported that the chromatographic fraction F5 of the aqueous extract of *Morinda morindoides* was well tolerated by kidney [26].

CONCLUSION

The aim of this study was to evaluate effect of the ethyl acetate fraction F1 of the hydroalcohol extract of *Morinda morindoides* (AcEF1) on the tissue and biomarkers of kidney in rabbit.

This study revealed only slight and no dose-dependent changes in uric acid and potassium. Overall, for its antidiarrheal, antifungal and antibacterial activities, this fraction did not harm renal tissue and would not be detrimental to renal function, thus, it would be well tolerated by kidney.

It would, however, be necessary to carry out further studies including cardiovascular tolerance as well as hematological investigations in order to obtain a fuller picture of the safety profile of the extract fraction.

ACKNOWLEDGMENT

The authors are grateful to Pr Ake of the Department of Botany and Dr Doukoure Brahima of Department of Anatomy-pathology of Medical Science, University of Cocody-Abidjan for their respective substantial contributions in botanical identification and collection of the plant and histopathological studies.

REFERENCES

[1] Kirby GC. Transaction of the Royal Society of Tropical Medicine and Hygiene 1996 ;90, 605-609.
[2] Hostesttmann K. and Marston A. Phytochem Rev 2002; 1:275-285.
[3] Bruhn JG, Holmestedt B. J Ethnopharmocology 1981 ; 405-430.
[4] Kambu K, Tona L, Luki N, Cimanga K, Uvoya A. Ann Pharm Fr 1990; 48 (5): 255-263.
[5] Tona L, Ngimbi NP, Tsakala M et al. Phytomed 1999; 6 (1): 59-66.
[6] Cimanga K, Hermans N, Apers S et al. J Natural Product 2003; 66: 97-102.
[7] Zirihi GN, Mambu L, Guede-Guina F, Bodo B, Grellier P. J Ethnopharmacol 2005; 98: 281-285.
[8] Cimanga K, Kambu K, Tona L et al. J Ethnopharmacol 2006; 107: 83-90.
[9] Bahi C, N'guessan JD, Guede-Guina F. Afr Biomed 2000; 5(1):11-18.
[10] Meite S, N'Guessan JD, Bahi C et al. Trop J Pharm Res 2009; 8 (3): 201-207

[11] Bagre I, Bahi C, Gnahoue G, Djaman AJ, Guede GF. J Sci Pharm biol 2007; 8(1): 15-23.
[12] Moroh JLA, Bahi C, Dje K, Loukou YG, Guede-Guina F. Bulletin de la société royale des sciences de Liège, 2008 ; 77 ; 44-61.
[13] Kamo ILBE. Etude des effects de BGG F1 sur le Coeur isolé de rat et la pression artérielle de lapin. DEA Biotechnologie, Université Abidjan-Cocody, 2008, 34p.
[14] Tra –Bi IO, Djyh BN, Bahi C, Meite S and N'guessan JD. Trop J Pharm Res; 2010; 9(6): 573- 579.
[15] Wyss M. Can J Pharmacol 1992; 70 (5): 733-734.
[16] Maurice L.Médecine thérapeutique 1999; 5(5) : 393-402.
[17] Guede-Guina F, Vangah-Manda M, Harouna D, Bahi C. J Ethnopharmacol 1993; 14 (2): 45-53
[18] 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.
[19] Founzegue AC, Adama C, N'Guessan JD, Koffi GK, Djaman AJ, Guede-Guina F. Sciences et Nature. 2007; 4(1): 37-43.
[20] Ramachandran SV. Circulation 2006; 113: 2335-2362.
[21] Yuegang Z, Chengjun W et al. Anal Sci 2008; 24: 1589-1592.
[22] Jagdish Kakadiya *et al*. JITPS. 2010; 1(6), 270-273.
[23] Corbett JV. Laboratory tests and diagnostic procedures with nursing diagnoses. 7th Ed.; 2008; 90-107.
[24] James S, Mitchel G. Physiology and disorder of water electrolytes and acid base metabolism. In: Carl AB, Edward R, David E .eds. Textbook of clinical chemistry and molecular diagnostics. 4th ed. New Delhi, Elsevier Inc 2006: 1747-1776.
[25] Eric P, Bruno A, Daniel R. Biologie Humaine : Anatomie, physiologie, santé Ed. Nathan, France. 1991; 383.
[26] DJYH B N. Etude de l'évolution de quelques paramètres sériques marqueurs de la biotolérance de trois phytomédicaments. Thèse de Doctorat 3è cycle, UFR, Biosciences, Université, Cocody-Abidjan ; 2003, 144p.