E3 Journal of Biotechnology and Pharmaceutical Research Vol. 4(2), pp. 22-28, February 2013
Available online at http://www.e3journals.org/JBPR
ISSN 2141-7474 © 2013 E3 Journals

Full Length Research Paper

Acute and subacute toxicity studies of "Hémodya", aqueous extract of three medicinal plants, used to fight against sickle cells disease in albino rats

Kotué, T. C[1*], Ndomou, M[2], Pieme, A. C[1], Oumdoum, P.C[3], Ngogang, Y. J[1]

[1]Laboratoire de Biochimie, de Physiologie et de Pharmacologie de la Faculté de Médecine et des Sciences Biomédicales/CHU, Université de Yaoundé 1, BP. 482 Yaoundé, Cameroun.
[2]Laboratoire de Biochimie de la Faculté des Sciences, Université de Douala, BP. 24157 Douala, Cameroun.
[3]Laboratoire de Biologie animale de la Faculté des Sciences, Université de Douala, BP. 24157 Douala, Cameroun.

Accepted 27 January, 2013

The present study was carried out to evaluate the acute and subacute toxicity of "Hémodya" a phytochemical drug used to fight against sickle cells disease. This drug is a combination of aqueous extract of three medicinal plants: Cassia siamea Lam (Euphorbiacea); Delonix regia, le flamboyant (Caesalpiniaceae) and Garcinia cowa Rox (Guttiferae) via the oral route in albino rats. For the acute toxicity test, a single dose administration of 5 g/kg bw. of "Hémodya" did not produce any toxic signs or deaths in rats. There were no significant differences ($p>0.05$) in the body and organ weights between control and treated groups. The (LD_{50}) of "Hémodya" was higher than 5 g/kg bw. In subacute toxicity study, no mortality and toxic signs were observed with the doses of 90, 180, 360 or 720 mg/kg bw. of "Hémodya" for 28 consecutive days. There were no significant differences in the body and organ weights between the control and treated animal of both sexes. Hematological analysis showed a significant difference ($p<0.05$) between hemoglobin and hematocrit estimations of the control and treatment groups with "Hémodya" after 28 days. However, no changes were observed in biochemical parameters of both sexes. Pathologically, neither gross abnormalities nor histopathological changes were observed. These results suggest that "Hémodya" did not produce toxic effects in albino rats. Collectively, these data demonstrate that it has a high margin of traditional drug safety.

Key words: Sickle cells disease, "Hémodya", medicinal plants, acute, subacute toxicity.

INTRODUCTION

Sickle cell disease (SCD), also called weakens sickle-shaped, is a genetic disease which affects the red blood cell (RBC) (OMS, 2006). On the biochemical and molecular level, sickle cell disease (SCD) is caused by a point mutation in the ß-globin gene of red blood cells (RBCs) hemoglobin. As result of this mutation, valine (a non-polar amino acid) is inserted into the ß-globin chain at the sixth position in place of glutamic acid (an electrically charged amino acid). The mutation in HbS causes the RBCs containing them to become stiff and sometimes sickle-shaped when they release their load of oxygen. The sickle cell mutation produces a 'sticky' patch on the surface of the β-chains when they are not complexed with oxygen (Arnal and Girot, 2002). Because other molecules of sickle cell hemoglobin also develop the sticky patch, they adhere to each other and polymerize into long fibres that cause the deformation of the normal disc biconcave RBC into a sickle shape. Small blood vessels are blocked by the clumping of sickled RBCs, preventing blood supply to various organs (OMS, 2006).

The pathological state appears at the individual homozygote and is characterized by a hemolytic anemia intersected with vaso-occlusive crises which are at the origin of the principal causes of death of sicklemians (Latoundji et al., 1991; Bunn, 1997).The prevalence of sickle cell disease (SCD) is 2% an average in Africa with

*Corresponding author. Email: ctkotue_bio@yahoo.fr. Tel: 237 77 22 34 18.

a life expectancy lower than 20 years (Gbadoé et al., 2001). In the other continents this prevalence is 0.02% (Galactéros, 2000).

In Africa, less than 50% of sickle cell patients attain the age of 5 years and less than18% arrive at the adulthood (Arnal and Girot, 2002). Faced with this pathological epidemiology and troubling mortality, the WHO recognizes this disease like a major public health problem in many countries. Moreover, the economic incidence of the sickle cell disease is of great importance in developing countries. This is why its treatment is out of reach of the populations of the sub-saharan Africa where the use of medicinal plants constitutes part of their culture and tradition. Indeed the African pharmacopeia, which is one of richest of the world and whose development is encouraged by WHO should be, from this point of view, valorized. Thus, the recourse to the local techniques used by traditional healers proves to be necessary. It is accordingly that Etamé in 1980 seriously engaged himself in phytotherapy research in Cameroon to fight against this disease. After almost 20 years of investigation, he identified in the central Region of Cameroon the medicinal plants with high potentials for the treatment of sickle cell disease. From this discovery, a drug called "Hémodya" was developed, obtained by decoction on the basis of the barks of 3 plants, and conditioned in the form of syrup. This syrup has been consumed for several years by the sickle cell patients. It results that the product invigorates the muscular system, oxygenates blood and supports the multiplication of RBCs (Etamé, 2000). It reduces the rate of hemoglobin S to the profit of the synthesis of A2 and F hemoglobins (Ngogang et al., 2003). This phytodrug used in Cameroon and in some central Africa countries may confer toxic properties to the sicklemians. It thus appears essential to carry out the evaluation of acute and subacute toxicity studies to have the limit of tolerance or safety margin for this product.

METHODOLOGY

"Hémodya",a decoction in syrup form containing 3 medicinal plants: Cassia siamea Lam (Euphorbiacea); Delonix regia, le Flamboyant (Caesalpiniaceae); Garcinia cowa Rox (Guttiferae) was provided by the “Pr. ETAME foundation” , P.O Box 14709 Yaoundé-Cameroon, Phone: +237 77765214/99275746, Email: etamewane@yahoo.com. The bottle contents were poured in conical flasks and were dehydrated to powder in oven at 45°C.

Laboratory animals

Male and female (nulliparous and nonpregnant) albino rats (aged 2 months, weighting 163–175g) were purchased from the animal facility of the laboratory of animal physiology of the Faculty of Science of the University of Yaoundé1. Animals were maintained under environmentally controlled conditions of 22 ± 3 ◦C and 12h light: 12h dark cycle. The animals had access to water and standard diet *ad libitum* and were kept in their cages for at least 5 days prior to dosing to allow for acclimatization in the laboratory conditions. Before the beginning of gavage of rats using gastric probe, the animals were deprived of food but not water throughout the night from 8 pm to 8 am. After this fasting period, each animal was weighed in order to calculate the dose of the product to be administered. Water was used as vehicle (2ml/100g bw). Four hours after the administration of the substance, the animals had free access to water and food. Every 3 days, the weights of all the animals were taken.

Acute experiment

This was performed according to the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals, No 425 (OECD, 2000). This is a directive of value, aimed to reduce the maximum number of animals necessary for experimentation and determination of lethal dose 50 (LD_{50}) of the substance tested (Bruce, 1985). The preferred rodent species is the female rats because are generally slightly more sensitive (Lipnick et al., 1995). Ten female rats were randomly divided into two groups: one control group and treated group. The control group received distilled water and the treated group received single dose of 5000 mg/kg body weight of "Hémodya" by gavage. Animals were observed for general behavioral and body weight changes, hazardous symptoms and mortality for a period of 14 days post-treatment. The lethal dose (LD_{50}) was estimated. Signs of toxicity and mortality were observed after the administration at the first, second, fourth and sixth hour and once daily for 14 days. At the end of the experiment, all rats were deprived of food, but not water throughout the night from 8 pm to 8 am, then were weighted and sacrificed by decapitation under light anesthesia with ether solvent for examination. The internal organs: liver, kidneys and pancreas were excised washed in Nacl (0.9%).

The toxicological effect of the extract was assessed on the basis of mortality, which was expressed as LD_{50}. If a test at one dose level of at least 5000 mg/kg body weight produced no compound related mortality, then a full study using three dose levels might not be necessary.

Subacute toxicity

This study was led according to the method recommended by the directive on the repetition of the amounts and toxicity described by OECD applicable No 407 on the rodents for one exposure time 28 days (OECD, 2006). A total of 84 animals; 42 males and 42 females were used. They were subdivided on the one hand into 7 groups of 12 animals (6 males and 6 females). The first group was the control group which received distilled water while the next four treatment groups received respectively 90 mg/kg/j (therapeutic dose of the drug); 180 mg/kg; 360 mg/kg and 720 mg/kg of "Hémodya" by daily oral gavage. The duration of treatment and observation for these first five groups was 28 days. The last two groups, called satellite groups, were divided into another control group and a treatment group that received the strongest dose (720mg/kg) daily for 28 days. These satellite groups were observed as above, with an additional 14 days post-treatment observation. The animals were examined each day out of the usual cage in order to detect any sign of toxicity.

Table 1. Effect of "Hémodya" on body and organ weights (g) of rats in acute toxicity

	Control (2ml water/100g)	«Hémodya» (5000mg/kg)
Body weight		
Initial	168± 3.114	170±1.898
Final	186±3.02	187±2.009
Organ weight		
Liver	6.62±0,207	6.62 ±0.321
Kidney	0.802±0.035	0.804±0.054
Pancreas	0.399±0.042	0.419±0.053

Data are expressed as mean ± s.d. (n=5). No statistical difference (p>0.05) between control and "Hémodya" group (p>0.05), (Schwart's test).

Blood analysis

The blood samples of each sacrificed animal were collected into EDTA (ethylene diamine tetra acetic acid) (0.5 ml) and dry non-EDTA centrifuge Huma tubes (1.5ml) by jugular vein samplaing. The EDTA blood was used for hematological study and red blood cell (HGB), leukocyte (WBC), hematocrit (HTC), and platelets (PLT) were assessed with an automatic hematological analyzer (Coulter Electronics ABX).

The serum was separated from the non-EDTA blood after centrifugation at 3000 rpm for 5 min and was assayed for alanine amino-transferase (ALT), aspartate amino-transferase (AST), serum urea, serum creatinine, total serum protein and serum α amylase by colorimetric methods (Cheesbrough,1991; Vasiliades,1976; Bergmeyer et al., 1978; Reitman and Frankel, 1957). Dosages were made using Architect (Abott ®) automation with Biolabo biochemical kits.

Tissue analysis

Liver, kidneys and pancreas were excised from dissected animals, washed with NaCl 0.9% (for removal of blood) and weighed, and stored in 10% formalin saline solution for histopathological analyses according to the techniques of bases described by Lison (1960).

Statistical analysis

The results are expressed as mean±S.d. The statistical significance between the control and treated groups was carried out with the software Sigma Start version 3.01A analysis software. The Schwartz Test was used to compare the means of the various parameters. It is a test of comparison of weak manpower (Schwartz, 1975). $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

This study was carried out to investigate the acute and subacute toxicity of "Hémodya" via the oral route in albino rats. "Hémodya" was administered at single dose of 5000 mg/kg bw (acute toxicity) and during 28 days (subacute toxicity test), at daily doses of 90 (therapeutic dose of the drug); 180; 360 or 720 mg/kg bw.

During the period of observation of the acute toxicity, none of the treated animals succumbed. No sign of toxicity such as the tremor, the change in step, excessive grooming, the convulsion, salivation, the diarrhea, the coma and the distress was raised. Their behavior was rather strengthened compared to the control group. At the end of 14 days, no death was recorded and no adverse effects of "Hémodya" indicating that the lethal dose 50 (DL_{50}) is higher than 5000 mg/kg. This therefore lends credit to the conclusion that the test substance is practically non-toxic or non-lethal after an acute exposure. According to Kennedy et al. (1986), substances that present LD_{50} higher than 5 g/kg via oral route may be considered practically non-toxic. Moreover, as per the Hodge and Sterner (1980) scale, its toxicity index was 5 thus almost non-toxic. This predicts a security for this product. Several medicinal plant extracts which have anti-sickling properties demonstrated low toxic effects these included *M. Lucida* and *N. leavis (*Joppa et al., 2008). The similar result was found with the ethanolic extract of the analgesic *Kaempferia galangal* whose DL_{50} was higher than 5g/kg (Kanjanapothi, 2004).

The test limit for acute oral toxicity is generally considered to be 5g/kg body weight. If no mortality is observed at this dose level, a higher dosage is generally not necessary (Hayes, 1989). There were no significant differences (p>0.05) between the control and treated groups in body and organ weights (Table 1).

Regarding the subacute toxicity, no death was recorded during the treatment period either in the control or treated groups. The animals did not show any changes in general behavior or other physiological activities. The body and organ weights of the male and female rats, which received "Hémodya" at 90 (therapeutic dose of the drug); 180; 360 or 720 mg/kg doses daily for 28 days, are given in Table 2. This result shows that there were no

Table 2. Effect of "Hémodya" on body and organ weights (g) of rats in subacute toxicity.

	« Hémodya» (mg/kg)				
	Control (2ml water/100g)	**90**	**180**	**360**	**720**
Body weight					
Male					
Initial	169±2.333	169±2	171±2	166±3.16	165±4.50
Final	206±4,1	208±3	209±1.233	204±4.73	207±5.09
Female					
Initial	170±1.31	171±1.41	170±2.55	166±4.44	168±1.01
Final	205±4.22	204±3.56	208±3.101	203±6.17	207±2.38
Organ weight					
Male					
Liver	6.982±0.711	7.07±0.794	6.68±0.536	7.202±0.692	6.88±0.56
Kidney	0.806±0.049	0.799±0.046	0.820±0.025	0.799±0.053	0.810±0.033
Pancreas	0.445±0.029	0.418±0.046	0.437±0.032	0.453±0.065	0.417±0.052
Female					
Liver	7.18±0.301	6.991±0.601	6.71±0.401	7.001±0.56	6.95±0.449
Kidney	0.789±0.01	0.801±0.036	0.785±0.015	0.811±0.044	0.803±0.011
Pancreas	0.444±0.016	0.425±0.028	0.434±0.011	0.432±0.018	0.420±0.026

Data are expressed as mean ± s.d. (n=6). No statistical difference (p>0.05) between control and "Hémodya" groups (p>0.05), (Schwart's test).

Table 3. Hematological values of rats treated with "Hémodya" in subacute toxicity.

	« Hémodya» (mg/kg)				
	Control (2ml water/100g)	**90**	**180**	**360**	**720**
Male					
HGB (g/dl)	14.914±0.939*	17.061±0.599*	16.143±0.653*	16.345±0.212*	16.275±0.228*
WBC (x10^6/ml)	6.448±0.038	6.450±0.049	6.446±0.049	6.445±0.072	6.444±0.064
HCT (%)	43.258±1.440*	45.108 ±2.619*	44.998±1.991*	45.27±0.560*	45.776±1.483*
PLT (x10^6/ml)	1039.8±83.280	1036.666±64.756	1032±73.872	1038±73.872	1040.22±19.483
Female					
HGB (g/dl)	15.005±0.831*	17.42±0.402*	16.217±0.456*	16.34±0,302*	17.00±0.09*
WBC (x10^6/ml)	6.339±0.04	6.428±0.036	6.399±0.029	6.401±0,551	6.51±0.041
HCT (%)	43.130±1.551*	45.654±2.215*	44.888±2.101*	44.799±1,702*	45.207±1.335*
PLT (x10^6/ml)	1052±44.5	1040±55.24	1039.16±48.517	1043.03±62,391	1043.527±44.2

Data are expressed as mean±s.d. (n=6)
* Significantly difference from the control (p<0.05), (Schwart's test).

significant differences (p>0.05) in the body and organ weights between control and treated animals in both sexes.

The hematological analysis (Table 3) showed a significant difference (p<0.05) for hemoglobin and hematocrit between control and treated animals of both sexes after 28 days. This difference emphasizes an increase in the rates of hemoglobin and hematocrit of the groups of animals which received "Hémodya". These rates were independent of the administered doses in both sexes. These results are in accordance with the study of Ngogang et al. (2003), who showed that "Hémodya" would act by enhancing the synthesis of hemoglobin. Similarly, results were obtained by Sanogo et al. (2008)

Table 4. Biochemical values of rats treated with "Hémodya" in subacute toxicity.

	« Hémodya» (mg/kg)				
	Control (2ml water/100g)	**90**	**180**	**360**	**720**
Male					
ALT (UI/l)	39.833±3.118	42.166±2.483	41.666±2.065	40.2±2.387	41.5±2.428
AST (UI/l)	65.166±4.337	67.166±6.853	66.333±5.278	64.2 ±3.033	66.166±8.256
Urea (g/l)	0.263±0.142	0.263±0.076	0.261±0.262	0.262±0.072	0.262±0.025
Creatinine (mg/l)	6.5±0.547	6.666±0.816	6.532±0.836	6.733±0.982	6.553±0.836
Total protein (g/l)	58.666±9.729	60±3.577	59.333±6.592	59±7.778	59.833±2.857
α amylase (UI/l)	4.950±0.432	5.050±0.504	5 ±0.544	5.084±0.626	5.112±0.663
Female					
ALT (UI/l)	40.526±2.088	41.235±2.117	39.912±3.012	40.99±2.06	41.523±2.353
AST (UI/l)	66.23±3.391	65.978±5.7425	66.036±4.167	65.791±7.145	65.055±5.742
Urea (g/l)	0.263±0.142	0.263±0.076	0.261±0.262	0.262±0.072	0.262±0.025
Créatinine (mg/l)	6.42±0.325	6.473±0.604	6.66±0.924	6.614±0.684	6.702±0.591
Total protein (g/l)	59.243±8.518	58.999±3.465	60.051±7.474	59.76±8.684	59.639±3.649
α amylase (UI/l)	5±0.255	5.232±0.301	5.249±0.635	5.115±0.853	5.018±0.446

Data are expressed as mean±s.d. (n=6)
No statistical difference (p>0,05) between control and "Hémodya" groups both sexes (Schwart's test).

Table 5. Body and organ weights (g) of rats treated with "Hémodya" in subacute toxicity (28 days followed by no treatment for 14 days).

	Control (2ml water/100g)	**«Hémodya» (720mg/kg)**
Body weight		
Male		
Initial	170±3.44	172±2.085
Final	219±4.764	222±2.25
Female		
Initial	169±4	173±3
Final	224±2.88	221±4.01
Organ weight		
Male		
Liver	7.71±0.632	7.121±0.682
Kidney	0.812±0.052	0.821±0.027
Pancreas	0.450±0.019	0.448±0.033
Female		
Liver	7.19±0.322	7.201±0.405
Kidney	0.718±0.05	0.818±0.02
Pancreas	0.453±0.011	0.451±0.043

Data are expressed as mean ± s.d. (n=6). No statistical difference (p>0.05) between control and "Hémodya" groups (p>0.05), (Schwart's test).

who demonstrated the increase of red blood cells in rats treated with the aqueous extract of *Argemone mexicana*, an anti-anaemic plant during 4 weeks. The consequence is the increase in the volume of the red blood cells which justifies the significant difference between the rate of hematocrit of the control group and those of the treated group. Analysis show no significant differences (p>0.05) for the other parameters of both sexes.

The biochemical profile of the treated and control groups are presented in Table 4. No statistically significant differences (p>0.05) were recorded in any of the biochemical parameters analyzed after 28 days in both sexes. .

Moreover, there was no effect on the levels of indicators of liver, kidney and pancreatic functions such as alanine amino-transferase (ALT), aspartate amino-transferase (AST), serum urea, creatinine, total protein and α amylase. This result demonstrated that "Hémodya" did not induce any damage to the liver, kidneys or pancreas and was earlier confirmed by the histological assessment of these organs (Hilaly et al., 2004).

In the satellite group, after 14 additional day observation, there was no late appearance of toxic effects. Moreover there was no observation of any significant difference (p>0.05) in the body and organ weights between control and treated animals (Table 5).

The hematological profile (Tables 6) obtained after 28 days followed by no treatment for 14 days were similar to those recorded after 28 days in both sexes.

No statistically significant differences (p>0.05) were recorded in any of the biochemical parameters analyzed after 28 days followed by no treatment for 14 in both sexes (Tables 7). The gross examination of the target

Table 6. Hematological values of rats treated with "Hémodya" in subacute toxicity (28 days of treatment followed by no treatment for 14 days).

	Control (2ml water/100g)	«Hémodya» (720mg/kg)
Male		
HGB (g/dl)	15.845±0.866*	16.985±0.401*
WBC (x10^6/ml)	6.891±0.055	6.774±0.039
HCT (%)	42.996±3.17*	46.28±1.922*
PLT (x10^6/ml)	1047±61.25	1046±51.251
Female		
HGB (g/dl)	14.862±0.472*	17.026±0.233*
WBC (x10^6/ml)	6.356±0.028	6.318±0.453
HCT (%)	43.02±1.343*	45.891±0.831*
PLT(x10^6/ml)	1043.09±36.8	1048±34.6

Data are expressed as mean±s.d. (n=6)
* Significantly difference from the control ($p<0.05$), (Schwart's test).

Table 7. Biochemical values of rats treated with "Hémodya" in subacute toxicity (28 days of treatment followed by no treatment for 14 days).

	Control (2ml water/100g)	«Hémodya» (720mg/kg)
Male		
ALT (UI/l)	39.956±2.353	40.381±2.066
AST (UI/l)	66.253±4.078	66.021±3.968
Urea (g/l)	0.262±0.015	0.261±0.099
Creatinine (mg/l)	6.501±0.715	6.472±0.0424
α amylase (UI/l)	5.113±0.552	5.173±0.304
Total protein (g/l)	59.538±4.013	58.02±3.013
Female		
ALT (UI/l)	41.332±3.01	41.463±3.036
AST (UI/l)	65.121±6.168	65.072±4.631
Urea (g/l)	0.262±0.253	0.263±0.031
Creatinine (mg/l)	6.478±0.402	6.648±0.750
α amylase (UI/l)	5.099±0.309	5.114±0.643
Total protein (g/l)	58.061±4.472	59.031±6.262

Data are expressed as mean±s.d. (n=6)
No statistical difference ($p>0.05$) between control and "Hémodya" groups both sexes (Schwart's test).

organs of the treated animals did not show significant changes in color and texture when compared with the control group. In addition, the microscopic analysis did not suggest histological alterations in any of the organs examined. This proves that "Hémodya" beyond the therapeutic dose induces neither necroses, hemorrhage nor inflammation of cells of the various organs of animals. These results are compatible with the biochemical markers obtained, which did not detect any dysfonction in the treated organ.

The overall data of this study suggest that the oral administration of "Hémodya" does not induce any toxic effects, which could stand as an assurance for the use of this "Hémodya" in folk medicine. Further investigation is needed to evaluate its chronic toxicity.

ACKNOWLEDGEMENTS

The authors are grateful to the coordinators of the Laboratory of Anatomy and Pathological Cytology, Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, for excellent technical assistance in the histopathology analysis.

REFERENCES

Arnal C, Girot R (2002). Drépanocytose chez l'adulte. Encycl. Méd. Chir. (Editions scientifiques et Médicales Elsevier SAS, Paris). Hématologie J. 13-006-D-16. pp. 15-26.

Bergmeyer HU, Scheibe P, Wahlefeld AW (1978). Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clin. Chem. 24: 58-73.

Bruce RD (1985). An Up-and-Down Procedure for Acute Toxicity Testing. Fundam. Appl. Tox. 5: 151-157.

Bunn HF (1997). Pathogenesis and treatment of sickle cell disease. N. Engl. J. Med. (337): 762-769.

Cheesbrough M (1991). Detection of bence jones protein in urine. In Medical Laboratory Manual for Tropical countries, (2nd ed.). ELBS with tropical health technology/Butterworth-Heinemann Ltd: Oxford, pp. 473-474.

Etamé E (2000). La découverte d'un nouveau traitement contre la drépanocytose à base des plantes médicinales du Cameroun, 13p.

Galactéros F *(*2000). Drépanocytose, Encyclop. Orphanet, p. 15.

Gbadoé AD, Atsou K, Agbodjan-Djossou OA, Tsolényanu É, Nyadanu M, Dogba AD, Assimadi JK (2001). Prise en charge ambulatoire des drépanocytaires: évaluation de la première année de suivi des patients dans le service de pédiatrie de Lomé (Togo). Bull. Soc Patho. Exot. (94): 101-105.

Hayes AW (1989). Principe and methods of toxicology. 2nd ed. Raven Press, New York, 34pp.

Hilaly JE, Israili ZH, Lyoussi B (2004). Acute and chronic toxicological studies of

Ajuga iva in experimental animals. J. Ethnopharmacol*.,* 91: 43-50.

Hodge AC, Sterner JH (1980). In études de toxicité: quelques données fondamentales (A. K. DONE) TEMPO MEDICAL Afrique N°7, 18p.

Joppa KM, Vovor A, Ekluk AA, Aklikokouk GM (2008). Effet de *Morinda Lucida Benth* (Rubiaceae) et de Newbouldia Leavis p. Beau v. (Bignoniaceae) sur la falciformation. Med. Trop., 68: 251-256.

Kanjanapothi D, Panthong A, Lertprasertsuke N, Rujjanawate C, Kaewpinit D, Sudthayakorn R, Choochote W, Chaithong U, Jitpakdi A, Pitasawa TB (2004). Toxicity of crude rhizome extrait of *Kaemferia galanga L*. (Prof Hom), J. Ethnopharmacol*.,* 90: 359-365.

Kennedy GL, Ferenz RL, Burgess BA (1986). Estimation of acute oral toxicity in rats by determination of the approximate lethal dose rather than the LD50, J. Appl. Toxicol. 6: 145-148.

Latoundji S, Anani L, Ablet ZI (1991). Morbidité et mortalité drépanocytaire au Bénin. Med. Afr. Noire*,* 38: 569-574.

Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP, Chu I, Goddard M, Segal L, Springer JA, Myers RC (1995). Comparison of the Up-and-Down, Conventional LD50 and Fixed Dose Acute Toxicity Procedures. Food. Chem. Toxicol., 33: 223-231.

Lison L (1960). Histochimie et cytochimie animales. Gauthiers-Villars, Paris, p. 842.

Ngogang J, Nkam M, Etame E, Nanfack P (2003). Hémodya sur certains paramètres biochimiques, Première rencontre de Biologie Spécialisée de Ouagadougou, 32p.

OECD (Organization for Economic Cooperation Development) Guideline, 425, (2000). Guideline document on acute oral toxicity. Environmental Health and Safety Monograph Series on testing and Assessment N° 24. Tox., 5: 151-157.

OECD (Organization for Economic Cooperation Development), Guideline, 407, (2006). Report of the validation of the Updated Test Guideline 407: Repeat dose 28-day Oral Toxicity Study in Laboratory Rats. Series on Testing and Assessment No 59, ENV/JM/MONO 26, 14pp.

OMS (2006). La drépanocytose dans la région Africaine : situation actuelle et perspective. Comité régional de l'Afrique 52ème session, 22p.

Reitman SN, Frankel S (1957). A colorimetric method for the determination of SGPT and SGPOT. Am. J. Clin. Pathol. 25: 56-62.

Sanogo R, Djimde A, Guirou C, Doumbia L, Maiga A, Doumbo O, Diallo D (2008). Etude de la toxicité subchronique du décocté d'Argemone mexicanal. Pharmacopée et Médecine Traditionnelle Africaines, 15: 26-31.

Schwartz D (1975). Méthodes statistiques à l'usage des médecins et des biologistes. Flammarion ed., 3ème édition, Paris, p. 290.

Vasiliades J (1976). Reaction of alkaline sodium picrate with creatinine: 1. Kinetics and mechanism of monocreatinine picric acid complex. Clin. Chem., 22: 1664-1671.