Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 6 (2): 149-157, 2011
ISSN 1816-496X / DOI: 10.3923/jpt.2011.149.157

Biological and Toxicological Effects of Aqueous Acetone Extract of *Cienfuegosia digitata* Cav. (Malvaceae) in Mice and Rats

[1]K. Konaté, [2]A. Souza, [2]M. Lamidi, [3]J.F. Djoba Siawaya, [2]F.H. Ella Mendene, [4]J. Millogo-Rasolodimby and [1]O.G. Nacoulma

[1]Laboratoire de Biochimie et Chimie Appliquées (LABIOCA), UFR/SVT, Université de Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso

[2]Institut de Pharmacopée et de Médecine Traditionnelle (IPHAMETRA), Centre National de la Recherche Scientifique et Technologique (CENAREST), Libreville-Gabon BP : 1156

[3]Laboratoire National de Santé Publique, Libreville-Gabon BP : 10736

[4]Laboratoire de Biologie et d'Ecologie Végétales, UFR/SVT, Université de Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso

Corresponding Author: Konaté Kiessoun, University of Ouagadougou, Burkina Faso Tel: 0024103357474, 0022670766237

ABSTRACT

Cienfuegosia digitata Cav., one specie of family of Malvaceae and a herbal plant well known in traditional medicine in Burkina Faso to treat infectious disease particularly hepatitis B. The present study was conducted to evaluate the biological and toxic effects of aqueous acetone extract of *Cienfuegosia digitata* Cav. in mice Swiss and albinos Wistar rats. In acute toxicity test, mice received doses of this extract by intraperitoneal route with LD_{50} value of 601.8 mg kg^{-1}. In sub-acute toxicity test, albinos Wistar rats were treat by gavage during 28 days with different doses of aqueous acetone extract of *Cienfuegosia digitata* Cav. (25, 50, 100 mg kg^{-1}). In biological parameters evaluations, the results varied widely in dose of extract and weight of rats and did not show clinical correlations. We undertook this study of extract in order to provide a scientific basis for the traditional use of *Cienfuegosia digitata* Cav. in traditional medicine. Present results of this study appeared to show the safety of acute and sub-acute toxicities of extract from of *Cienfuegosia digitata* Cav. which can therefore be continuously used with safety in traditional medicine. Statistical studies revealed that there is a low significant difference in body and organ weights and biological parameters between control group and the treated assay groups ($p<0.01$ or $p<0.05$).

Key words: *Cienfuegosia digitata,* LD_{50}, toxicity, biological parameters, mice, Wistar rats

INTRODUCTION

Medicinal plants constitute an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80% of the rural population depends on them as primary health care (Akinyemi, 2000). Plants have been used as sources of remedies for the treatment of many diseases since ancient times and peoples of all continents especially Africa have this old tradition because the diverse culture is a rich source of traditional medicines. Many African's countries uses traditional medicine for their health needs (Ouedraogo *et al.*, 2007). In West Africa, new drugs are not often affordable. Thus, up to 80% of the population uses medicinal plants as remedies (Hostettmann and Marson, 2002). In Burkina Faso, traditional medicine is

mainly based on the use of medicinal plants (Tapsoba and Deschamps, 2006). Therefore, the study of toxicity activity of these medicinal plants is useful for the enhancement of traditional medicine, as well as for the development of new therapeutic molecules (Farnsworth, 1994). It is on this basis that researchers keep on working on medicinal plants in order to develop the best medicines for physiological uses (Usman and Osuji, 2007). Medicinal plants take up an important place in scientific research and many controlled trials have been done to investigate their plant substances efficacy and the results indicate that some Burkinabe medicinal herbs may work in various diseases. Among them, *Cienfuegosia digitata* Cav. Malvaceae specie found in several parts of the country is very used in popular folk medicine for treatment of several diseases. Ethnobotanical investigations in the central region of Burkina Faso have shown that *Cienfuegosia digitata* Cav. is used frequently and widely in traditional medicine to treat various kinds of diseases such as infectious diseases in children and is very widely used for the treatment of liver diseases for many years in Burkina Faso particularly in hepatitis B virus treatment, malaria, fever, pain, variola, antibacterial, anti-viral activities and hepatoprotective (Nacoulma, 1996). Phytochemical analysis of this plant demonstrated the presence of saponosides, coumarins, steroids, tannins, polyphenols and alkaloids (Nacoulma, 1996). Despite the extensive use of these plants in traditional health care, the literature provides little information regarding their toxicity so that the toxicities effect of this very used are unknown. The objective of the present study was to assess the toxicological study and evaluation of biochemical and haematological parameters of aqueous acetone extract of *Cienfuegosia digitata* Cav. In short, the study consisted in administrating extract by intraperitoneal route on the albinos Wistar rats and to monitor the progression of biological parameters.

MATERIALS AND METHODS

Plants material: *Cienfuegosia digitata* Cav. was collected in August 2008 in Gampela, 25 km east of Ouagadougou, capital of Burkina Faso. The plants were botanically identified by Millogo-Rasolodimby from the plants Biology Department of the University of Ouagadougou. A voucher specimen was deposited at the Herbarium of the Laboratoire de Biologie et d'Ecologie Végétale, UFR/SVT of University of Ouagadougou.

Preparation of extracts: Fifty grams of powdered plant material was extracted with 80% aqueous acetone (500 mL) in 1/10 ratio (w/v) for 24 h under mechanic agitation (SM 25 shaker, Edmund BÜHLER, Germany) at room temperature. After filtration, acetone was removed under reduced pressure in a rotary evaporator (BÜCHI, Rotavopor R-200, Switzeland) at approximately 40°C and freeze-dried by a being Telstar Cryodos 50 freeze-dryer. The extract residues were weighed before packed in waterproof plastic flasks and stored at 4°C until use.

Animals: We used male mice Swiss NMRI (20-30 g) and adult albinos Wistar (160-170 g) of both sex coming from University of Yaoundé (Cameroun). The animals were housed in cage under controlled conditions of 12-h light/12-h dark cycle and 25°C. They all receive pellets food enriched with protein 20% and water *ad libitum*.

Toxicity studies

Acute toxicity study in mice: Healthy male and female Swiss mice (20-30 g) were randomly divided into 7 groups (1 control group and 6 treated assay groups) of 6 animals (3 male and 3

female). They deprived of food, but not water 15 h prior to the administration of the test suspension. The control group received water containing 10% dimethylsulfoxide (DMSO) administered by intraperitoneally. The aqueous extract acetone of *Cienfuegosia digitata* suspended in 15% DMSO was administered intraperitoneally at doses of 200, 300, 500, 750, 1000 and 1500 mg kg^{-1}. The general behavior of the mice was observed at 120 min after the treatment. The animals were observed for morbidity and mortality once a day for up 14 days, with food and water *ad libitum*. The number of survivors after the 14 days period was noted. The toxicological effect was assessed on the basis of mortality, which was expressed as the median lethal dose (LD_{50}) (Miller and Tainer, 1944).

Sub-acute toxicity study in albinos Wistar rats: Wistar rats were divided into 4 groups of 6 animals (3 males and 3 females). Body weight was (160-170 g). The first group served as control and received water containing DMSO 10%. The remaining groups (group 2; group 3; group 4) received three dose levels of the *Cienfuegosia digitata* extract (25, 50 and 100 mg kg^{-1}) suspended in 10% of DMSO, administered orally by gavage daily for a period of 28 days. Body weight was measured weekly and the animals were observed daily for signs of abnormalities throughout the study. At the end of a 28 day period, the animals were deprived of food for 15 h. Blood samples were collected by cardiac puncture for biochemical and haematological examinations and selected organs were carefully dissected and removed for weighing.

Blood analysis: Blood samples were collected by cardiac puncture in three tubes for haematology, glucose and serum biochemistry. The blood samples with heparin and without anticoagulant were centrifuged at 30000 rpm for 5 min to obtain plasma or serum. Plasma was used to determine glucose by Trinder (1969) and Burn and price (1985) method and the serum for other biochemical parameters such as aspartate aminotransferase (AST) and alamine aminotransferase (ALT) determined according Schumann *et al.* (2002) and according Schumann *et al.* (2002), alkaline phosphatase (ALP) estimated by German Society for Clinical Chemistry (1972) and Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974) methods, creatinine (Fossati *et al.*, 1983), uric acid (Fossati *et al.*, 1980), blood Urea Nitrogen (BUN) according Fawcett and Scott (1960), triglycerides (Fossati and Prencipe,1982), total cholesterol (Allain *et al.*, 1974), total bilirubin and direct bilirubin determined according to Sherwin and Thompson (2003). All these biochemical parameters were measured by Selectra XL Vital Scientific (Elitech Group Company).

Haematological analyses were performed on whole blood, using the automatic counter (Mindray Auto hematology Analyser BC-5500) to evaluate the following parameters such as erythrocyte count (RBC), haemoglobin, haematocrit, platelet count, leucocyte count (WBC), neutrophilis, basophilis, eosinophilis, lymphocytes, monocytes, MCV, MCH and MCHC.

Organ and animals weights: The body weights of animals were measured weekly and at the end of a 28 day period, the animals were deprived of food for 15 h. After the collection of blood samples by cardiac puncture for biochemical and haematological examinations, organs such as heart, lungs, stomach, liver, kidneys in rats were carefully dissected and removed for weighing.

Statistical analysis: The data were expressed as Mean±Standard deviation (SD) of six determinations (n = 6). Results were analyzed by one-way ANOVA followed by Dunnett's t-test using Prism 4 software. The level of significance was accepted at $p \leq 0.05$.

RESULTS

Acute toxicity study in mice: The effect of intraperitoneal treatment of the aqueous acetone extract from *Cienfuegosia digitata* on mortality, LD_{50} value. The value of LD_{50} is 601.8 mg kg^{-1} for intraperitoneal administration. No significant difference in body weight gain of the treated assay groups over the period of observation. No statistical difference was observed between the organ weights in the control and the intraperitoneal route groups.

Sub-acute toxicity study in rats

Body weight: We noticed no significant difference in body weight gain between control group and the test groups ($p>0.05$). However, there is also an increase in animal weight as a function of treatment time (in weeks). In the fourth week, there was a significant difference in body weight gain between the test groups and the control group ($p<0.01$). We note a decrease in weight of animals, the results are summarised in Table 1.

Organ weights: Table 2 shows the effects of *Cienfuegosia digitata* Cav. extract on the weights of some vital body organs in rats. The weights of liver (25 and 50 mg kg^{-1}; $p<0.05$) and heart (25 and 50 mg kg^{-1}; $p<0.01$ and $p<0.05$) decreased significantly compared to the control group (DMSO 10%). However there is no significant difference between the other vital body organs weights of the treated assay groups and the control group ($p>0.05$).

Haematological analyse: The effects of *Cienfuegosia digitata* extract on the haematological parameters are shown in Table 3. The treated rats showed significant differences from the control group respectively in MCHC (50 mg kg^{-1}; $p<0.01$), basophils (50 mg kg^{-1}; $p<0.01$) and monocytes

Table 1: Animal weights (g) with time of treatment

Groups	1st day	1st week	2nd week	3rd week	4th week
1	165.33±3.78	189.33±13.69	209.00±12.43	229.33±16.93	229.67±14.87
2	164.17±4.12^{ns}	175.33±3.62*	172.67±10.48**	176.67±11.18**	171.00±15.72**
3	164.83±3.60^{ns}	167.00±6.26**	167.00±13.24**	171.33±29.18**	162.67±5.96**
4	165.67±3.78^{ns}	162.67±9.18**	183.33±11.54**	194.00±5.59**	201.67±8.12**

Values are Mean±SEM (n = 6) one-way ANOVA followed by Dunnett's t-test: Compare all vs. control: $^{ns}p>0.05$, *$p<0.05$, **$p<0.01$ compared with control. Group 1: Control, rats received 10% DMSO, Group 2: Rats received 10% DMSO with extract (25 mg kg^{-1} b.wt.), Group 3: Rats received 10% DMSO with extract (50 mg kg^{-1} b.wt.), Group 4: Rats received 10% DMSO with extract (100 mg kg^{-1} b.wt.)

Table 2: Effects of aqueous acetone extract of *Cienfuegosia digitata* on the weights (g) of organs of rats

Groups	Left kidney	Right kidney	Stomach	Lungs	Liver	Heart
1	0.68±0.04	0.72±0.08	2.28±1.05	1.47±0.22	6.63±0.76	0.72±0.05
2	0.68±0.08^{ns}	0.64±0.08^{ns}	2.13±0.35^{ns}	1.18±0.22^{ns}	5.26±0.05*	0.59±0.03**
3	0.65±0.06^{ns}	0.66±0.06^{ns}	1.84±0.38^{ns}	1.33±0.16^{ns}	5.29±0.58*	0.62±0.03*
4	0.68±0.07^{ns}	0.67±0.06^{ns}	2.85±0.14^{ns}	1.69±0.54^{ns}	5.57±1.21^{ns}	0.64±0.09^{ns}

Values are Mean±SEM (n = 6) one-way ANOVA followed by Dunnett's *t*- test: : Compare all vs. Control: $^{ns}p>0.05$, *$p<0.05$, **$p<0.01$ compared with control. Group 1: Control, rats received 10% DMSO, Group 2: Rats received 10% DMSO with extract (25 mg kg^{-1} b.wt.), Group 3: Rats received 10% DMSO with extract (50 mg kg^{-1} b.wt.), Group 4: Rats received 10% DMSO with extract (100 mg kg^{-1} b.wt.)

Table 3: Effects of aqueous acetone extract of *Cienfuegosia digitata* on the biochemical parameters in the plasma and the serum of rats

Biochemical parameters	Group 1	Group 2	Group 3	Group 4
Glucose (mmol L^{-1})	6.550±0.16	4.570±0.19**	3.400±0.11**	3.650±0.06**
Uric acid (mmol L^{-1})	0.210±0.09	0.186±0.12^{ns}	0.177±0.008^{ns}	0.416±0.017**
Urea nitrogen (mmol L^{-1})	9.620±0.3	12.230±2.77*	9.150±0.06^{ns}	9.370±0.04^{ns}
Creatinine (mmol L^{-1})	0.041±0.006	0.040±0.007^{ns}	0.020±0.004*	0.012±0.003*
AST (UI L^{-1})	82.500±2.74	107.670±2.25**	116.000±3.23**	91.330±3.14**
ALT (UI L^{-1})	37.500±13.69	90.000±10.95**	62.500±13.69**	55.000±5.48*
ALP (ALP L^{-1})	74.500±2.74	103.130±2.33**	107.220±1.96**	80.500±2.74**
Triglycerides (mmol L^{-1})	0.870±0.40	0.630±0.07^{ns}	0.480±0.03**	0.250±0.05**
Total cholesterol (mmol L^{-1})	1.970±0.36	2.400±0.66^{ns}	1.550±0.05^{ns}	1.750±0.05^{ns}
Total bilirubin (mmol L^{-1})	0.100±0.03	0.118±0.06**	0.157±0.003**	0.233±0.001**
Direct bilirubin (mmol L^{-1})	0.002±0.00	0.005±0.00**	0.011±0.001**	0.012±0.00**

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase, Values are Mean±SEM (n = 6) one-way ANOVA followed by Dunnett's t- test: Compare all vs. control: ^{ns}p>0.05,*p<0.05, **p<0.01 compared with control. Group 1: Control, rats received 10% DMSO, Group 2: rats received 10% DMSO with extract (25 mg kg^{-1} b.wt.), Group 3: Rats received 10% DMSO with extract (50 mg kg^{-1} b.wt.), Group 4: Rats received 10% DMSO with extract (100 mg kg^{-1} b.wt.)

Table 4: Effects of aqueous acetone extract of *Cienfuegosia digitata* on the haematological parameters on whole blood of rats

Haematological parameters	Group 1	Group 2	Group 3	Group 4
WBC (10^3 μL^{-1})	12.02±3.02	11.00±1.10^{ns}	10.64±2.25^{ns}	10.64±1.76^{ns}
RBC (10^6 μL^{-1})	7.38±0.61	7.91±0.36^{ns}	7.18±0.83^{ns}	6.51±0.79^{ns}
Eosinophil (%)	2.33±1.86	2.00±1.79^{ns}	0.33±0.52^{ns}	2.00±0.89^{ns}
Lymphocyte (%)	77.33±7.17	72.67±10.67^{ns}	77.00±6.20^{ns}	71.50±8.22^{ns}
Neutrophil (%)	26.50±4.93	25.33±10.60^{ns}	22.33±5.96^{ns}	26.00±8.76^{ns}
Monocyte (%)	2.00±0.00	0.33±0.82**	0.33±0.52**	0.50±0.83**
Basophil (%)	0.13±0.21	0.10±0.15^{ns}	1.10±0.59**	0.30±0.27^{ns}
Haemoglobin (g dL^{-1})	14.00±1.03	15.40±0.56*	14.10±0.88^{ns}	13.75±0.71^{ns}
Haematocrit (%)	41.30±2.94	44.60±1.57^{ns}	39.43±3.91^{ns}	37.03±4.53^{ns}
MCV (μm^3)	56.03±0.68	56.40±1.08^{ns}	55.07±2.15^{ns}	56.90±2.33^{ns}
MCH (pg)	19.00±0.24	19.43±0.23^{ns}	19.77±1.25^{ns}	19.97±0.36^{ns}
MCHC (g dL^{-1})	33.90±0.09	34.50±0.31^{ns}	35.87±1.43**	35.10±1.21^{ns}
Platelet (x10^3 μL^{-1})	958.00±34.13	968.00±95.15^{ns}	957.00±152.63^{ns}	851.67±32.97^{ns}

WBC: leucocyte count; RBC: erythrocyte count; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration. Values are Mean±SEM (n = 6) one-way ANOVA followed by Dunnett's t-test: Compare all vs. control: ^{ns}p>0.05, *p<0.05, **p<0.01 compared with control, Group 1: Control, rats received 10% DMSO, Group 2: Rats received 10% DMSO with extract (25 mg kg^{-1} b.wt.), Group 3: Rats received 10% DMSO with extract (50 mg kg^{-1} b.wt.), Group 4: Rats received 10% DMSO with extract (100 mg kg^{-1} b.wt.)

(25 mg kg^{-1}; p<0.01, 50 mg kg^{-1}; p<0.01 and 100 mg kg^{-1}; p<0.01). There is no significant difference between the control group (10% DMSO) and the other the treated assay groups (p>0.05).

Biochemical analyses: Table 4 shows the effects of *Cienfuegosia digitata* Cav. extract on the biochemical parameters. Glucose (25, 50 and 100 mg kg^{-1}; p<0.01; p<0.01 and p<0.01), uric acid (100 mg kg^{-1}; p<0.01), urea nitrogen (25 mg kg^{-1}; p<0.05), creatinine (50 and 100 mg kg^{-1}; p<0.05 and p<0.05), AST (25 , 50 and 100 mg kg^{-1}; p<0.01), ALT (25, 50 and 100 mg kg^{-1}; p<0.01; p<0.01 and p<0.05), ALP (25, 50 and 100 mg kg^{-1}; p<0.01), triglycerides (50 and 100 mg kg^{-1}; p<0.01 and p<0.01), total bilirubin (50 and 100 mg kg^{-1}; p<0.01 and p<0.01) and direct bilirubin (50 and 100 mg kg^{-1}; p<0.01 and p<0.01) were significantly changed in the treated assay groups compared

to the control group (10% DMSO). For the other biochemical parameters however, there is no significant difference between the control group (10% DMSO) and the other treated assay groups ($p>0.05$).

DISCUSSION

Nowadays, it is noteworthy that traditional medicine is gaining popularity in developing countries. Medicinal plants are often believed to be harmless because they are natural and are commonly used for self-medication without supervision. This increase in popularity and the scarcity of scientific studies on their safety and efficacy have raised concerns regarding toxicity and adverse effects of these remedies (Saad *et al*., 2006). These products of plants contain bioactive principles with the potential to cause adverse effects (Bent and Ko, 2004).

The results of the present study indicated that the extract of *Cienfuegosia digitata* is low poisonous. During the 14 day period of acute toxicity evaluation, some signs of toxicity were observed, but they were all quickly reversible. According to Diez (1989), pharmacological substances whole LD_{50} is less than 5 mg kg^{-1} b.wt. are classified in the range of highly toxic substances, those with a LD_{50} between 5 mg kg^{-1} b.wt. and 5000 mg kg^{-1} b.wt. are classified in the range of moderately toxic substances and those with the lethal dose is more than 5000 mg kg^{-1} b.wt. not toxic. In this fact, if we refer to this classification we could say that the extract of *Cienfuegosia digitata* are moderately toxic and would be regarded as being safe or of low toxicity (Clarke, 1977).

For the sub-acute study at doses of 25, 50 and 100 mg kg^{-1} body weight during 28 day period, we noted any change in animal behaviour or mortality. Changes in body weight and internal organ weights could be due to the adverse side effects. According Raza *et al*. (2002) and Teo *et al*. (2002), weight loss is a simple and sensitive index of toxicity after exposure to toxic substance. We notice a low variation between animal weights and their internal organs compared with control group. This suggests that the extract of *Cienfuegosia digitata* is of low toxicity.

For the results of biochemical parameters, we notice a variation between the different doses administered but this variation is low. There is a low significant difference between the control group (10% DMSO) and the other treated assay groups ($p<0.05$ or $p<0.01$). Many research works reported that some factors can be useful in differentiating a significant change from control values, from a treatment-related effect. This difference is less likely to be an effect to treatment if: there is no obvious dose response; it is due to finding in one or more animals that could be considered outlier; it is within normal biological variation (Lewis *et al*., 2002). At this, such changes do not suggest that the extract of *Cienfuegosia digitata* produced toxicity in the treatment period. Biochemical evaluation is important, because kidney and liver toxicity has been reported the use of phytotherapeutic products (Corns, 2003; Hilaly *et al*., 2004; Isnard *et al*., 2004; Saad *et al*., 2006). In the present study, creatinine, urea and uric acid determinations were critical as markers of kidney function (Newman and Price, 1999). There is not much significant differences in uric acid, creatinine and urea comparatively to the control group ($p<0.01$ or $p<0.05$). This was also confirmed by the variation of kidney weights (right kidney and left kidney). There is no significant difference between control group and the other treated assay groups ($p>0.05$). Among the parameters evaluated, AST, ALT and ALP are considered markers of liver function (Tolman and Rej, 1999; Hilaly *et al*., 2004). There is not much differences in AST, ALT and ALP comparatively to the control group ($p<0.01$ or $p<0.05$). The results revealed relationship between these enzymatic

markers and liver function and this was demonstrated by the variation of liver weight. We notice there is no significant difference between control group and the other treated assay groups ($p>0.05$).

However, high levels of glucose and uric acid in control group may be explained by the food of rats which contains proteins and some sugar. Several studies have revealed that xanthine oxidase is the enzyme responsible for the formation of uric acid from the purines hypoxanthine and xanthine and is responsible for the medical condition known as gout (Meda *et al*., 2010). In this fact, the decrease in uric acid during the treatment because *Cienfuegosia digitata* has xanthine oxidase inhibitory properties (Nacoulma, 1996).

For the haematological parameters, it appears that basophils and monocytes changes did not appear to be related to the treatment with *Cienfuegosia digitata* extract, because they showed no dose-response relationship. Moreover, in general there was a decrease in the rate of RBC in all rats. This can be explained by the phenomenon of haemolysis probably which could be due to the stress caused by collection of blood in rats. Blood samples were collected by cardiac puncture that could explain the stress in rats.

CONCLUSION

The toxicological and biological effects obtained in this study seem be interesting for the therapeutic use of *Cienfuegosia digitata*. The low toxicity evidenced by LD_{50} value suggests a wide margin of safety for therapeutic doses. In sub-acute study, some effects were observed but there were no relevance of serious signs or significant changes in animal weights, effect of extract on animal organs, haematological and biochemical parameters. Briefly, these toxicity studies suggest that the extract of *Cienfuegosia digitata* is safe.

ACKNOWLEDGMENTS

The authors are grateful to the France Embassy in Burkina Faso/EGIDE-France for the mobility scholarship. The authors thank also the technicians, Ph.D. student from laboratories of pharmacology and phytochemistry of IPHAMETRA (Libreville-Gabon) and National Laboratory of Public Heath (Libreville-Gabon) for their collaboration in this research.

REFERENCES

Akinyemi, B., 2000. Recent concept in plaque formation. J. Clin. Pathol., 30: 13-16.

Allain, C.C., L.S. Poon, C.S.G. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.

Bent, S. and R. Ko, 2004. Commonly used herbal medicines in the United States: A review. Am. J. Med., 116: 478-485.

Burn, J.M. and C.P. Price, 1985. Measurement of blood glucose. Ann. Clin. Biochem., 22: 327-327.

Clarke, E.G.C., 1977. Veterinary Toxicology. Cassel and Collier Macmillan Publishers, London, pp: 268-277.

Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology, 1974. Recommended methods for the determination of four enzymes in blood. Scand. J. Lab. Invest., 3: 291-306.

Corns, C.M., 2003. Herbal remedies and clinical biochemistry. Ann. Clin. Biochem., 40: 489-507.

Diez, J., 1989. Toxicology: Basic Principles and Chemical Impact. In: Pharmacology: Fundamental Principles to Therapeutic Applications, Slatkine, M. (Ed.). Academic Press, Geneve, pp: 33-44.

Farnsworth, N.R., 1994. Ethnopharmacology and Drug Development. In: Ethnobotany and the Search for New Drugs, Ciba Foundation Symposium 185, Prance, G.T. and J. Marsh (Eds.). John Wiley and Sons, Chichester, pp: 42-59.

Fawcett, J.K. and J.E. Scott, 1960. A rapid and precise method for determination of urea. J. Clin. Pathol., 13: 156-159.

Fossati, P., L. Prencipe and G. Berti, 1980. Use of 3,5-dichloro-2-hydroxy-benzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymatic assay of uric acid in serum and urine. Clin. Chem., 26: 227-231.

Fossati, P. and L. Prencipe, 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28: 2077-2080.

Fossati, P., L. Prencipe and G. Berti, 1983. Enzymic creatinine assay: A new colorimetric method based on hydrogen peroxide measurement. Clin. Chem., 29: 1494-1496.

German Society for Clinical Chemistry, 1972. Recommendations of the German society for clinical chemistry. Standardisation of methods for the estimation of enzyme activities in biological fluids. Experimental basis for the optimized standard conditions. Z. Klin. Chem. Klin. Biochem., 10: 281-291.

Hilaly, J., Z.H. El Israili and B. Lyoussi, 2004. Acute and chronic toxicological studies of Ajugaiva in experimental animals. J. Ethnopharmacol., 91: 43-50.

Hostettmann, K. and A. Marston, 2002. Twenty years of research into medicinal plants: Results and perspectives. Phytochem. Rev., 1: 275-285.

Isnard, B.C., G. Deray, A. Baumelou, M. Le Quintree and J.L. Vanherweghem, 2004. Herbs and the kidney. Am. J. Kidney Dis., 44: 1-11.

Lewis, R.W., R. Billington, E. Debryune, A. Gamer, B. Lang and F. Carpanini, 2002. Recognition of adverse and nonadverse effects in toxicity studies. Toxicol. Pathol., 30: 66-74.

Meda, N.T.R., A. Lamien-Meda, M. Kiendrebeogo, C.E. Lamien, A.Y. Coulibaly, J. Millogo-Rasolodimby and O.G. Nacoulma, 2010. *In vitro* antioxidant, xanthine oxidase and acetylcholinesterase inhibitory activities of *Balanites aegyptiaca* (L.) Del. (Balanitaceae). Pak. J. Biol. Sci., 13: 362-368.

Miller, L.C. and M.L. Tainter, 1944. Estimation of the ED50 and its error by means of logarithmic probit graph paper. Proc. Soc. Exp. Biol. Med., 57: 261-264.

Nacoulma, O.G., 1996. Medicinal plants and their traditional uses in Burkina Faso. Ph.D. Thesis, University of Ouagadougou, pp: 328.

Newman, D.J. and C.P. Price, 1999. Renal Function and Nitrogen Metabolites. In: Tietz Textbook of Clinical Chemistry, Burtis, C.A. and E.R. Ashwood (Eds.). W.B. Saunders Co., Philadelphia, ISBN-10: 0721656102, pp: 1204-1270.

Ouedraogo, Y., I.P. Guissou and O.G. Nacoulma, 2007. Biological and toxicology study of aqueous root extract from *Mitragyna inermis* (Willd oktze) Rubiaceae. Int. J. Pharmacol., 3: 80-85.

Raza, M., O.A. AI-Shabanah, T.M. El-Hadiyah and A.A. Al-Majed, 2002. Effect of prolonged vigabatrin on hematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. Scientia Pharmaceutica, 70: 135-145.

Saad, B., H. Azaizeh, G. Abu-Hijleh and S. Said, 2006. Safety of traditional Arab herbal medicine. Evid. Based Comp. Alt. Med., 3: 433-439.

Schumann, G., R. Bonora, F. Ceriotti, G. Ferard and C.A. Ferrero *et al.* 2002. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 4. Reference procedure for the measurement of catalytic concentration of alanine aminotransferase. Clin. Chem. Lab. Med., 40: 718-724.

Sherwin, J.E. and C. Thompson, 2003. Liver Function. In: Clinical Chemistry: Theory, Analysis Correlation, Kaplan, L.A., A.J. Pesce and S.C. Kazmierczak (Eds.). 4th Edn., Mosby Inc., EDS, St. Louis, USA., pp: 493.

Tapsoba, H. and J.P. Deschamps, 2006. Use of medicinal plants for the treatment of oral diseases in Burkina Faso. J. Ethnopharmacol., 104: 68-78.

Teo, S., D. Stirling, S. Thomas, A. Hoberman and A. Kiorpes and K. Vikram, 2002. A 90-day oral gavage toxicity study of D-methylphenidate and D, L methylphenidate in sprague-dawley rats. Toxicology, 179: 183-196.

Tolman, K.G. and R. Rej, 1999. Liver Function. In: Tietz Textbook of Clinical Chemistry, Burtis, C.A. and E.R. Ashwood (Eds.). W.B. Saunders Co., Philadelphia, pp: 1125-1177.

Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., 2: 185-161.

Usman, H. and J.C. Osuji, 2007. Phytochemical and *in vitro* antimicrobial assay of the leaf extract of *Newbouldia laevis*. Afr. J. Trad. Complement. Altern. Med., 42: 476-480.