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TOXICOLOGICAL AND ANTIHYPERTENSIVE EFFECTS OF *ZINGIBER OFFICINALE* ROSCOE AQUEOUS EXTRACT ON WISTAR RATS.

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ABSTRACT

This study indicated pharmacological basis for the use of *Zingiber officinale* in some diseases. In mice, aqueous extract of *Zingiber officinale* showed any toxicity at the doses from 100 to 5000 mg/kg of body weight; subacute toxicity revealed non-significant values of hematological and biochemical parameters at the doses 200 and 400mg/kg of body weight, compared to control. This plant may not affect blood and some organs. Then, aqueous extract of *Zingiber officinale* decreases normal blood pressure at the doses from 10^{-3} to 5mg/kg of body weight. This lowering can reach 60 mmHg for high dose of 5mg/kg of body weight. Acetylcholine causes also blood pressure decrease corresponding to *Zingiber officinale* aqueous extract at dose of 10^{-1}mg/kg. Norepinephrine increased blood pressure at ≈ 200 mmHg; aqueous extract of *Zingiber officinale* lowers this hypertension at normal blood pressure from cumulative dose to 1mg/kg of body weight. Our results suggest that *Zingiber officinale* possesses hypotensive, antihypertensive and innocuousness properties and probably explains its use in hypertension and alimentary.

KEYWORDS: *Zingiber officinale*, hypotensive, antihypertensive, subacute, innocuousness.

INTRODUCTION

Zingiber officinale Roscoe (Zingiberaceae) commonly known as ginger has been used in the world millions years ago. It is used in healer prescriptions (Akram, 2011). In west Africa, rhizomes of this plant are used as drink known "gnamakoudji" (Amani *et al*., 2007). Hypertension is the most recurrent of cardiovascular disease. Its prevalence is crescent in developing countries. According to Fourcade *et al*. (2007) we would have 150 millions of hypertensive people in 2025. In Burkina Faso, economic stake led people to use medicinal plants (Ministère de la santé, 2004). This study is to evaluate toxicological and antihypertensive effects of the aqueous extract of this plant on normotensive mice and rats respectively and to understand the mechanism of action.

I. MATERIAL AND METHODS

I.1. Plant collection

Fresh rhizomes of *Zingiber officinale* were bought at the local market at Ouagadougou (Burkina Faso, West Africa). This plant was identified by Dr Ouédraogo, Department of Botany, University Joseph KI-ZERBO. The herbarium specimens have been deposited in this department; identification number is 16874 and species number is 6822.


A


B

Figure 1: *Zingiber officinale* rhizome (A) and plant (B).

I.2. Preparation of plant extract

The shade dried rhizomes of *Z. officinale* were used to prepare aqueous extract. Four hundred grams of rhizome powder were macerated in three liters (3L) of deionized water for 24h at room temperature (30°C) with shaking. Extract macerated was filtered and centrifuged (2000 tours/min) and floating was dried. Extract mass and extraction yield were determined.

I.3 Animals

Naval Medical Research Institute (NMRI) mice (28-30 g) and Wistar rats (250 – 300 g) of two sexes were used in this study. The animals were fed with standard diet and kept 24 ± 2°C, 60 ± 10% humidity and submitted to a 12h light/dark cycle with free access to food and water. All animals procedures were strictly within national laws and guidelines (the animals used in accordance with the local ethic committee of University Joseph KI-ZERBO for the use and care of animals).

I.4 *In vivo* blood pressure measurement

An invasive method was used. The animals were anaesthetized with urethane (40%).The trachea was exposed and canulated to facilitate spontaneous respiration. Two catheters (PE-50) were implanted in the right jugular vein and left carotid artery for drug administration and recording of arterial blood pressure, respectively. Blood pressure and heart rate were monitored using a pressure transducer connected to the Harvard isolated preamplifier, and displayed on one channel of an oscillograph. Then, windpipe (for breathing derivation), carotid (for blood pressure recording) and jugular vein (for extract injection) were isolated. A catheter was filled with heparin saline solution and connected to collector of pressure (Belemnaba et *al*., 2014).

After equilibrium period (20 min), 0.1 mL of saline solution (0.9 %) was injected to jugular vein. The same quantity was used for others tested substances. Blood pressure was returned at equilibrium between different injections. This test was repeated at least five times to validate results.

After injection of saline solution, aqueous extract of *Zingiber officinale* was injected at increasing doses (10^{-3} to 5 mg/kg pc). Others substances such as acetylcholine (Ach, 10^{-5} mg/kg) and norepinephrine (NE, 10^{-3}mg/kg) were injected to have control responses. Atropine (Atr) at dose of 10^{-4} mg/kg was used to determine AEZO mechanism.

I.5 Toxicity test

I.5.1 Acute toxicity test

According to the method described by Bayala, (2005), seventy two female mice were used and eighteen groups were made, each group contains four animals. The animals were fasted for twenty four hours with free access to water and water was withdrawn ten hours before the experiment. Different groups were treated with aqueous extract in increasing doses (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 and 5000 mg/kg). One hour after administration of the extract, the animals were allowed to eat. They were observed regularly at the period of 1, 24, 48 and 72 hours for lethality or toxic effects as behavioral changes.

I.5.2. Sub acute toxicity

This study was made with female rats. Eighteen animals were fasted for three groups, each group contains six animals. Two groups were treated daily, during 28 days, at the same time with aqueous extract in increasing doses (200 and 400 mg/kg) respectively. The reference group was treated with saline solution (0.9 %) on the same period. Animal's behaviors were observed daily, weight and food consummation were measured each week.

At the end of this treatment, animals were fasted for twenty four hours with free access to water. The blood was collected by heart puncture for biochemical and hematological tests. Some organs such as heart, spleen, liver, kidney and lung were removed, weighed and kept into formol (10 %) for histopathological test.

I.6. Drugs

Norepinephrine, atropine and acetylcholine were purchased from Sigma chemicals Co. St Louis, MO, USA. NaCl 0.9 % was purchased from Ouagadougou pharmaceutical office (Burkina Faso). The aqueous extract, acetylcholine, atropine and norepinephrine were dissolved in saline solution. All solutions were freshly prepared on the day of experiment.

I.7. Statistical analysis

All data were expressed as means ± standard error of the means (S.E.M.). Statistical analysis was conducted using two ways ANOVA followed by Dunnett post, test. A value of $p < 0.05$ was considered statistically significant. All analyses were performed with Graph Pad Prism 5.00 (Graph Pad Software, San Diego, CA, USA).

II. RESULTS

II. 1 Plant extraction

Table-1.

Extract	Yield (%)	Dry Extract mass obtained (g)	Extract mass recovered (g)
Aqueous extract	14.0	33.23	32.25 g

The residual humidity content is 5.1% and the yield of the plant extract is 14%.

II.2. *In vivo* test of blood pressure

Aqueous extract of *Zingiber officinale* decreases blood pressure in dose-dependent manner. This decrease reached 60 mmHg at dose of 5mg/kg. Acetylcholine causes also blood pressure decrease corresponding to *Zingiber officinale* aqueous extract at dose of 10^{-1}mg/kg (fig.2).

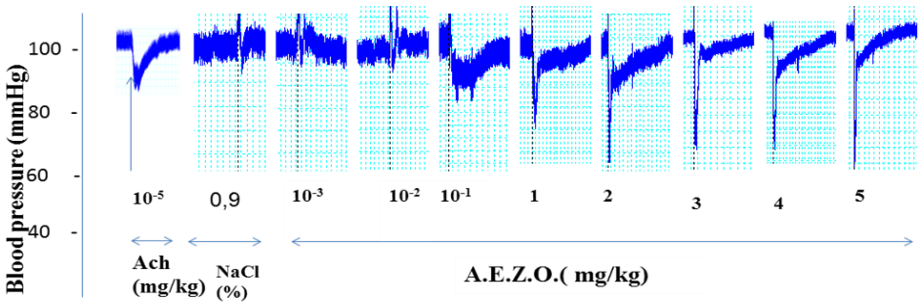

Figure 2: Blood pressure decrease recording.

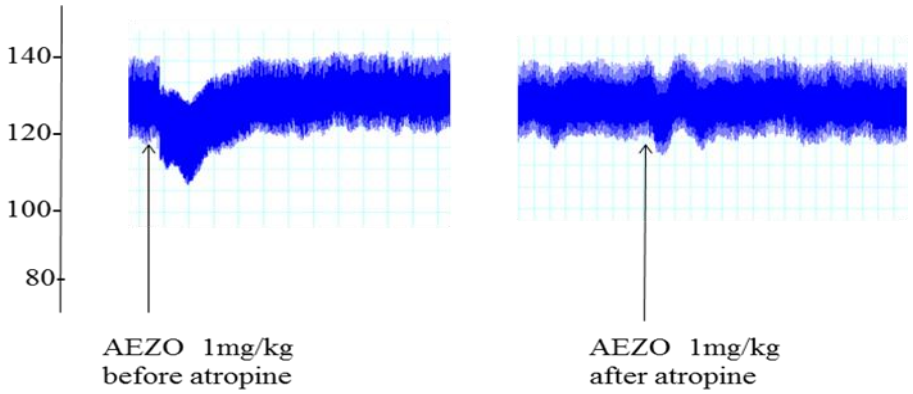

Figure 3: Blood pressure recording in presence and absence of atropine.

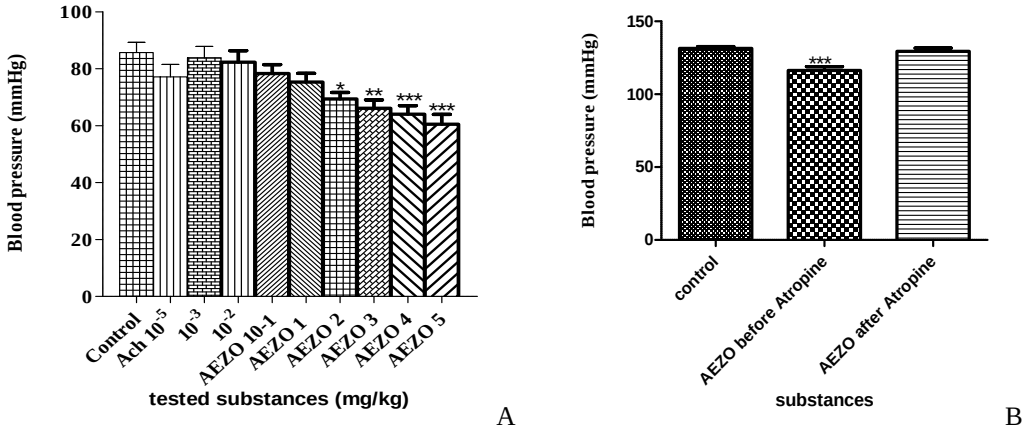

Figure 4: A: Blood pressure lowering induced by Ach and AEZO. B: Blood pressure lowering in presence and absence of atropine.

*P< 0.01; **P< 0.001; ***P < 0.0001.

AEZO induces blood pressure lower significantly at doses from 2mg/kg to 5mg/kg; p.c. (fig.4 A) Blood pressure lowering before atropine is significant but this lower is not significant after atropine application (fig.4 B).

When the blood pressure induced by NE reached a plateau, cumulative applications of the extract of *Z. officinale* decrease the blood pressure in dose dependent fashion (fig.5).

II.3. Antihypertensive test with norepinephrine

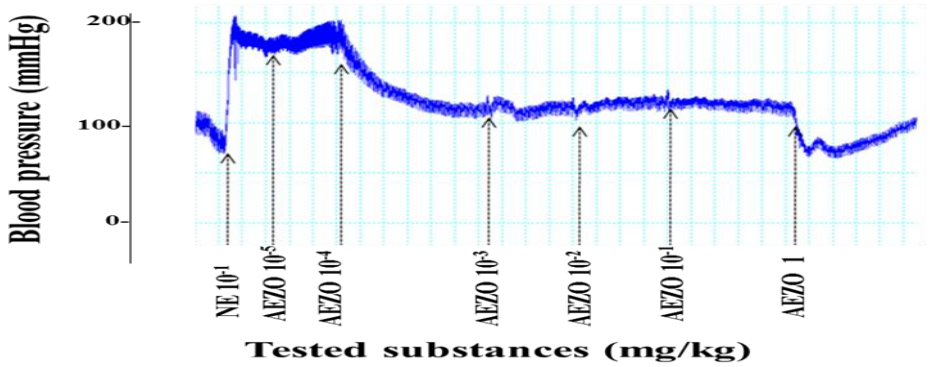

Figure 5: Effect of AEZO on blood pressure induced by norepinephrine.

II.4. Toxicological studies

II.4.1. Acute toxicity

Oral acute toxicity showed that there are any deaths for all doses tested even high dose of 5000 mg/kg of body weight. However tested animals present drowsiness sign and food lowering consumption during the first time after administration.

II.4.2. Sub acute toxicity

Zingiber officinale aqueous extract did not cause change on different studies parameters. Animals which are been tested by AEZO at doses of 200 and 400 mg/kg, do not present modification of haematological parameters values (table 1) compared to control. Biochemical parameters (table 2) do not present equally no significant modification of AEZO at doses 200 and 400 mg/kg compared to control.

Organs weight of heart, spleen, liver, lung and kidney are not changed after AEZO treatment (table 3) compared to control. Animal's weight (figure 5) has changed each week but not significant. However, from first week to fourth week, their weights have changed significantly compared to control.

Table 2: Haematological parameters between treated and control groups rats after treatment with AEZO.

Parameters	Control	AEZO 200	AEZO 400
RBC 10^6/mm^3	7.90±0.43	8.11±0.34	7.75±0.61
HB g/dl	14.55±0.85	15.03±0.70	14.46±0.51
HcT %	40.36±2.04	41.7±1.75	40.4±2.03
VGM %	51±0.89	51.5±1.22	52.16±2.31
PLA 10^3/mm^3	716±69.63	692.5±57.18	694.33±37.47
WBC 10^3/mm^3	1.61±0.57	2.05±0.28	2.43±1.19
LYM %	79.76±8.66	86.7±3.40	89.53±4.67
MON %	6.33±2.42	4.31±1.89	3.28±1.40
NEU %	12.88±6.34	8.03±2.10	6.18±2.84
BAS %	0.46±0.48	0.26±0.25	0.08±0.13
EOS %	0.55±0.57	0.68±0.83	0.91±1.35

RBC: Red Blood Cells; HB: hemoglobin; HcT: hematocrit; PLA: Platelets; WBC: White Blood Cells; LYM: Lymphocytes; MON: Monocytes; NEU: neutrophils; BAS: basophils; EOS: eosinophils.

Table 3: Biochemical parameters between treated and control groups rats after treatment with AEZO.

PARAMETERS	control	AEZO 200	AEZO 400
CREATININE (µmol/L)	41.16±6.46	42.5±6.05	50±7.80
UREA (mmol/L)	8.55±1.04	9.9±0.69	7.68±0.54
TOTAL PROTEIN (g/L)	69±4.14	67.83±5.34	68.5±2.81
AST (U/L)	340±174.92	325.5±124.69	333.33±137.25

ALT (U/L)	65.33±19.93	62.5±7.71	57.33±12.20
CHOLESTEROL (mmol/L)	1.71±0.30	1.63±0.46	1.73±0.45
Total Bilirubin (µmol/L)	2.2±0.66	1.96±0.29	2.13±0.30

Table 4: Organs weight between treated and control groups rats after treatment with AEZO.

Organs(g)	control	AEZO 200	AEZO 400
HEART	0.62± 0.08	0.60±0.07	0.62±0.05
SPLEEN	0.46±0.10	0.49±0.02	0.50±0.04
LUNG	0.94±0.12	0.86±0.05	0.96±0.14
LIVER	5.54±0.57	5.56±0.39	6.15±0.66
KIDNEYS	1.42±0.16	1.46±0.18	1.34±0.13

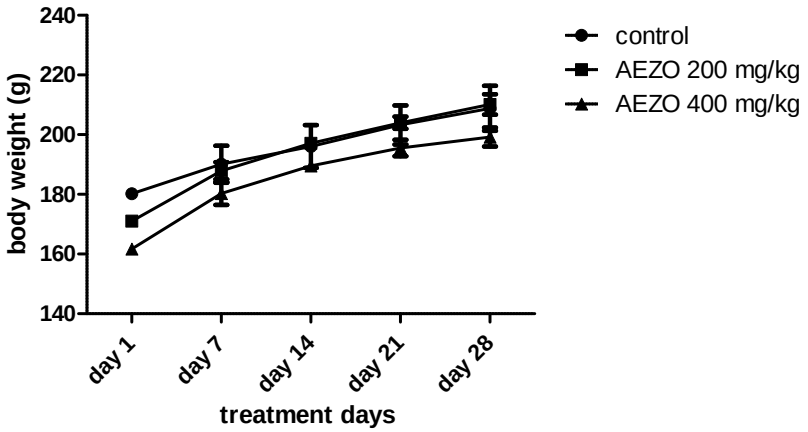

Figure 6: Body weight between treated and control groups during 28 days.

III. DISCUSSION

Zingiber officinale aqueous extract and acetylcholine decrease blood pressure in dose-dependent manner in anaesthetized normotensive rats. Saline solution (NaCl 0.9 %) has no effect on animal's blood pressure and comforts its vehicle role. Indeed, cardiovascular system is controlled by sympathic and parasympathic systems. Sympathic stimulation causes blood pressure increase but parasympathic stimulation decreases blood pressure (Bestel et *al*., 1999). Calcium entry in cell facilitates acetylcholine release in synaptic crack. Acetylcholine fixation on postsynaptic receptors leads to its stimulation. But acetylcholine can settle on presynaptic receptors to modulate its liberation. In this case acetylcholine would be settled in presynaptic receptors to involve blood pressure lowering. AEZO decreases blood pressure, but this decrease is reduced by atropine. With atropine (10^{-4} mg/kg), blood pressure decrease induced by AEZO (1mg/kg) is completely reduced. Atropine, a muscarinic receptor antagonist inhibited hypotension induced by Ach. Indeed, when Ach is fixed on muscarinic receptor M_2, it inhibits adenyl cyclase and cAMP formation on presynaptic receptors and decreases functioning of cardiac muscle (Dangoumau et *al*., 2006). Then, this result suggests that *Zingiber officinale* aqueous extract contains some components which may be able to cause blood pressure lowering by their fixation on muscarinic receptors of acetylcholine. In this case our result is in agreement with those obtained for the aqueous leaf extract of *Sclerocarya birrea* on rat (Belemtougri et *al*., 2007) and the aqueous leaf extract of *Moringa olifeira* on rat (Somé et *al.*, 2015). In vascular smooth muscle, contraction is induced by calcium concentration (Rico et *al*., 1990). Phenylephrine (NE) induced calcium release *via* IP_3 receptors on sarcoplasmic reticulum. Indeed, NE stimulates α_1 adrenergic receptors leading to convert phosphatidylinositol to inositol 1, 4, 5- triphosphate (IP_3) on postsynaptic receptors which increase calcium release from intracellular store and increasing of cardiac muscle functioning (Dangoumau et *al*., 2006). AEZO relaxes hypertension induced by NE and could lower blood pressure by blocking calcium release from this channel. Our results are in agreement with those of Siddiqi et *al*. (2014) and Khan et *al*. (2014). Phytochemical studies of *Zingiber officinale* show the presence of many substances such as flavonoids, sesquiterpenes, manganese that would be able to involve in blood pressure lowering (Nacoulma/Ouédraogo, 1996). Flavonoids are phenolic compounds which contribute to reduce cardiovascular risk by their antioxidant activity (Mpondo Mpondo et *al*., 2012). *Zingiber officinale* contains shoagol and gingerol which are the main responsible of the most of physiological phenomenons. Adjagba et *al*. (2015) studies show that flavonoids could be involved in the antihypertensive effects of *Tridax procumbens*. Indeed, oxidative stress is defined as an imbalance between the production of

reactive oxygen species (ROS) and antioxidant network. Our lifestyle such as smoking, alcoholism, obesity and also our inadequate diet contribute to increase the production of ROS in our organism. *Zingiber officinale* could lower blood pressure by antioxidant effects of flavonoids (Haleng et *al*., 2007). Stoilova et *al*. (2007) have proved an antioxidant activity of ginger extract in lipid peroxidation inhibiting.

Toxicological studies of herbal extract in animals are commonly used to assess potentials health risk in human caused by intrinsic adverse effects of chemical compounds of plants extracts (Ashafa et Olunu, 2011). In this study *Zingiber officinale* acute toxicity shows a LD_{50}>5000 mg/kg. According to Hodge and Sterner (1943) scale and World Health Organization, this drug may be classed without risk. Ghayur and Gilani (2007) studies showed that aqueous and methanolic extracts of *Zingiber officinale* do not involved mortality at doses up to 5000mg/kg/p.c. Our results are in agreement with their results. Subacute toxicity study shows any mortality during treatment time. Animal's weight in tested groups does not increase significantly compared to control group. Haematological and biochemical parameters do not present significant values between control and tested groups likewise that organ weight. Assessment of haematological parameters can be used to determine the extent of deleterious effects of compounds including plant extracts on animal's blood constituents (Ashafa et *al*., 2009). The no significant change in white blood cells (WBC) suggests that the overall immune function has not been compromised. WBC or leucocytes are vitally important in the disposal of damage and ageing tissues and immune responses which protect body from infection and cancers cells proliferation (Ashafa et *al.*, 2011). The non significant effect of the extract in red blood cells (RBC) may be an indication that the balance between the rate of production and destruction of the blood corpuscles was not altered (erythropoeisis).The effect of extract on platelets is not significant and may show any adverse effects on the oxygen carrying capacity of the blood as well as thrombopoeitin (McLellan et *al*., 2003). Aqueous extract of *Zingiber officinale* studies on biochemical parameters suggests that values have not change significantly compared to control. It may indicate that extract has not negative effects on liver, heart and kidneys. Indeed, ALT and AST (transaminases) elevation is noticed in the case of hepatic cytolyse and muscular necrosis. Transaminases are activated in liver, heart and muscles. It pass in serum at hepatic or muscular cytolyse case. ALT increases the most in liver diseases but AST increase the most in muscular necrosis. Creatinine and urea elevation indicates kidney insufficiency. Creatinine is daily eliminated in urine at fixe quantity according to muscular weight of subject. So there is correlation between creatinine and glomerular flow; when the glomerular flow lowers, high quantity of glomerular filtration leads to also high quantity of creatinine. As to urea, its mixture is asked to objectivized renal insufficient. The hypercholesterolemia is a factor of artherosclerosis. Bilirubin dosage confirms icterus diagnostic; bilirubin is damage produce of hemoglobin which is released in plasma and carried to liver. Hemoglobin damage increasing involves a hyperbilirubinemia (Caquet, 2010). Our results suggest a protective effect of the plant extract of liver and kidney.

CONCLUSION

Our study showed that *Zingiber officinale* aqueous extract exhibited hypotensive and/or antihypertensive effect on normotensive rat blood pressure *in vivo*. Its action on NE induced hypertension on rat blood pressure may be done on isolated organs such as aortic rings and atria to confirm the involvement of α_1 adrenergic receptors on these organs because blood pressure is the product of peripheral resistance and cardiac output. Further investigation will be done to precise the nature of active principles which cause antihypertensive effects on rats *in vivo*. The observed effects seem to justify its traditional use to treat hypertension and its utilization as beverage.

REFERENCES

1. Akram M, Shah IM, Usmanghan K, Mohiuddin E, Asif ASM, Shah SMA, Ahmed K, Shaheen G, *Zingiber officinale* Roscoe (A Medicinal Plant). Pakist. J. Nutrit., 2011; 10: 399-400.
2. Amani G, Tetchi FA, Coulibaly A, Propriétés physico-chimiques de l'amidon de gingembre (*Zingiber officinale* Roscoe) de Côte d'Ivoire. Tropicultura, 2004; 22(2): 77-83.
3. Fourcade L, Paule P, Mafart B, Arterial hypertension in sub-Saharan Africa: Update and perspectives. Med. Trop., 2007; 67: 559-567.
4. Ministère de la Santé, Document cadre de politique nationale en matière de médecine et pharmacopée traditionnelles. Burkina Faso, 2004; 18.
5. Belemnaba L, Nitiema M, Traoré S, Somé N, Traoré A, Ouedraogo S, Guissou IP, Recherche de plantes à potentialité antihypertensive dans la biodiversité du Burkina Faso. Pharm. Med. Trad. Afr., 2014; 17(1): 33-40.
6. Bayala B, Progestative activity and œstrogenic activity of *Holarrhena florinbunda (G.* Don) Durand et Schinz (Apocynaceae), a medicinal plant of traditional pharmacopea of Burkina Faso. Thesis, Univ. Ouaga, 2005; 93.
7. Bestel J, Clairambault J, Medigue C, Monti A, Sorine M, Le système cardiovasculaire et sa régulation par le système nerveux autonome: modélisation et mesures automatiques. Biologie & santé, 1999; 9: 65-92.
8. Dangoumau J, Moore N, Molimard M, Fourrier-reglat A, Latry K, Haramburu F, Miremont-salame G, Titier K, Pharmacologie générale. Département de pharmacologie Université Victor Segalen Bordeaux 2. ISBN:2-909176-24-X, 558, 2006.

9. Belemtougri GR, Dzamitika SA, Ouedraogo Y, Sawadogo L. Effects of water crude leaf extract of *Sclerocarya birrea* (A. Rich) Hochst (Anacardiaceae) on normotensive rat blood pressure. J. Biol. Sci., 2007; 7: 570 – 574.
10. Somé AA, Belemtougri RG, Ouedraogo Y, Traoré A, Ouedraogo S, Effects of water crude leaf extract of *Moringa oleifera* Lam (Moringaceae) on normotensive rat blood pressure and isolated duodenum. J. Phys. Pharm. Adv., 2015; 5(10): 771-778. ISSN: 2251-7677.
11. Rico L, Alonso M J, Salaices M, Marin J, Pharmacological dissection of Ca^{2+} channels in the rat aorta by Ca^{2+} entry modulators. Pharmacol, 1990; 40: 330-342.
12. Siddiqi HS, Majeed A, Gilani AH, Pharmacological basis for the medicinal use of *Wrightia tinctoria* in hypertension and dyslipidemia. J. cardiovasc. pharmacol., 2014; 64: 151-163.
13. Khan M, Khan AU, Rehman N, Gilani AH, Blood pressure lowering effect of *Morus alba* is mediated through Ca^{++} antagonist pathway. Int. J. Pharmacol., 2014; 10(4): 225-230.
14. Nacoulma/Ouédraogo OG, Medicinal plants and traditional medicinal practices in Burkina Faso: case of central plateau. Thesis, Univ. Ouaga, tome I, tome II, 1996; 328 & 338.
15. Mpondo-Mpondo E, Dibong SD, Yemeda CFL, Priso RJ, Ngoye A, Les plantes à phénols utilisées par les populations de la ville de Douala. J. Plant & Sciences, 2012; 15(1): 2083-2098.
16. Adjagba M, Awede B, Nondichao K, Lagnika L, Osseni R, Darboux R, Laleye A, Antihypertensive activity of different fractions of *Tridax Procumbens* crude aqueous extract in wistar rats. J. Phys. Pharm. Adv, 2015; 5(9): 713-719.
17. Haleng J, Pincemail J, Defraigne JO, Charlier C, Chapelle JP, Le stress oxydant. Rev. Med. Liège, 2007; 62(10): 628-638.
18. Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S, Antioxidant activity of a ginger extract (*Zingiber officinale*). Food Chem., 2007; 102: 764–770.
19. Ashafa AT, Olunu OO, Toxicological evaluation of ethanolic root extract of *Morinda lucida* (L.) Benth. (Rubiaceae) in male wistar rats. J. Nat. pharm., 2011; 2: 108-114.
20. Hodge HC, Sterner JH, Determination of substance acute toxicity by LD_{50}. American Industrial Hygiene Association, 1943; 10: 93.
21. Ghayur MN, Gilani HA, Inhibitory activity of ginger rhizome on airway and smooth muscle preparations. Eur. Food Res. Technol., 2007; 224: 477-481.
22. Ashafa AOT, Yakubu MT, Grierson DS, Afolayan AJ, Effects of aqueous leaf extract from the leaves of *Chrysocoma ciliate L.* on some biochemical parameters of wistar rats. Afr. J. Biotechnol., 2009; 8: 1425-1430.
23. Ashafa AOT, Sunmonu TO, Afolayan AJ, Effects of leaf and berry extracts of *Phytolacca dioica* L. on haematological and weight parameters of wistar rats. Afr. J. Pharm. Pharmacol., 2011; 5(2): 150-154.
24. McLellan SA, McLellan DBL, Walsh TS, Anemia and red blood cell transfusion in the critically ill patient. Blood Rev., 2003; 17: 195-208.
25. Caquet R, 250 examens de laboratoire, prescription et interprétation. Elsevier Masson SAS. 11, ISBN: 978-2-294-71033-9, 432, 2010.