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Evaluation of Sub-acute Oral Toxicity of Hydroethanolic Leaves Extract of *Solenostemon monostachyus* (Lamiaceae) in Mice.

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ABSTRACT

The present study was carried out to evaluate subacute toxicity of a hydroethanolic extract of *Solenostemon monostachyus* (Esomo) in mice. Four groups of six mice were orally treated with Esomo at 0, 500, 1000 and 2000 mg /kg b.w. for 28 days and hematological, biochemical parameters and histopathological study carried out after experimental period. The control group of mice received saline solution.Subacute administration of Esomo did not exert any significant variation on body and organs weights, on food and water intake. This treatment did not show any change in hematological parameters such as erythrocytes and leukocytes count, hemoglobin concentration, hematocrit and mean corpuscular volume. Histopathological analysis and clinical blood chemistry parameters revealed no toxic effects of the extract. However, at doses of 1000 and 2000 mg/kg b.wEsomo induced thrombocytopenia. Our results exhibit that the extract of *S. monostachyus* is not devoid of toxicity.

Keywords: *Solenostemon monostachyus*, subacute toxicity, haematological parameters, clinical blood chemistry and histopathological study.

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INTRODUCTION

Medicinal plants have been used for generations for their reputed qualities to treat several diseases. The safety record of plants is often well established and the traditional medicinal applications associated with the remedy are usually supported by modern clinical studies[1].

However, many of these medicinal plants contain chemical constituents that could cause harmful effects to human if taken in large quantities. *Carica papaya* leaves, for example, contain cyangenic glycosides which form cyanide. This compound, at high concentration, can produce adverse reactions[2]. Also, medicinal plants are administered in most disease conditions over a long period of time without a proper dosage monitoring and consideration of toxic effects that might result from such a prolong use[3]. It is then essential to know adverse reactions that could arise from prolong use of natural products, in order to estimate adequately their suitability for use by human.

Solenostemon monostachyus P. Beauv. (Lamiaceae) is a plant widely distributed in Ivory Coast. Leaves are eaten as a potherb. Previous studies on the chemical composition of fresh leaves have led to identify: water, proteins, lipids, glucids, calcium, phosphate[4]. The most important essential oil in the leaves is β-pinene[5]. This plant has numerous medicinal uses. In East region of Ivory Coast, the leaves were employed as oxytocic for parturient to make easier deliveries and also to treat hypertension by Abbey traditional medicine practitioners[6]. Our research group has previously investigated the phytochemical and antioxidants properties of this plant[7]. In this context, its antioxidant activity was described and related with the presence of terpens, coumarins, flavonoids, tannins and polyphenols. Structures of six coleons (diterpenoids) from this plant were elucidated[8].

Lethal dose often varies depending on the method of administration, for instance many substances are less toxic when administered orally than when intraperitonially administered. Acute toxicity of hydroethanolic extracts of *S. monostachyus* leaves was studied by two administration routes. Acute oral study realized by Onu[9] had showed a LD_{50} of 537 mg/kg p.c. and the LD_{50} found with the same extract was 1900 ±0.1 mg/kg p.c. intraperitonially[7]. As a measure of toxicity, median lethal dose is somewhat unreliable and results may vary greatly between testing facilities due to factors such as the genetic characteristics of the sample population, animal species tested, environmental factors and mode of administration[10]. Chronic toxicity study is required to avoid possible adverse effects of the extract.

The aim of the present study was to assess subacute toxicity of hydroethanolic extract from leaves of *S. monostachyus* in mice.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *S. monostachyus* (Lamiaceae), collected in June (rain season) from farms specialized in growing plants for medicinal purposes, was identified and authenticated by Pr Aké-Assi Laurent, expert botanist. Voucher specimens number 8217 were preserved and catalogued in the Herbarium of the Centre National de Floristique (Abidjan, Cote d' Ivoire)

Preparation of the extract

The methods were previously described by Kpahé *et al.*[11]. The collected plant material was dried at room temperature and powdered. 750 g of the fine powder was extracted with 3 L of 70 % ethanol by maceration during 24 hours under magnetic shaker. The suspension was filtered. The filtrate collected was evaporated. The final extract yielded (0.5%, w/w) and was stored at 5 °C for further use.

Experimental animals

Experimental procedures and protocols used in this study were approved by theEthical Committee for animal research of Félix Houphouët Boigny University, Abidjan. These guidelines were in accordance with the internationally accepted principles for laboratory use and care[12,13].

Female Swiss mice (28-35 g) were cared for and treated according to the principles for the care and use of laboratory animals for biomedical research. They were bred in Animal house of Nutrition and Pharmacology Department, UFR Biosciences (Abidjan, Cote d'Ivoire). These mice were kept in temperature- controlled environment (25 ± 2°C) with a 12 h light-dark cycle and had free access to water and standard diet.

Sub-acute toxicity studies were performed according to Organization for Economic Co-operation and Development guidelines and principles on good laboratory practice[14].

Subacute toxicity test

Swiss female mice (28-35 g) were used for subacute toxicity evaluation. Four groups of six mice were employed to test the drug. Control groups received saline solution (0.9 %) and were, used as reference. The three other groups (I, II, III) were treated with Esomo extract *per os*, in doses of 500, 1000 and 2000 mg/kg b.w., respectively during 28 days. Food and water intake were measured daily. After 28 days, each animal was anaesthetized with diethylether. Blood samples were collected via retro-orbital puncture in EDTA-coated tubes and used for hematological and biochemical analysis[15,16]. Animals were sacrificed by decapitation and liver and kidney were kept for histological analysis. The body and organs weights were determined.

Hematological parameters

Hematological parameters were determined using a blood autoanalyser (Sysmex kx-21 N). Erythrocytes number, leukocytes number, hematocrit, hemoglobin, platelets and mean corpuscular volume (MCV) were determined.

Biochemical estimations

Blood sample collected in the heparinized tubes were centrifuged at 3000 rpm for 10 minute. The serum separated was analyzed for various parameters such as Serum Glutamic oxaloacetic Transaminase, Serum Glutamic Pyruvic transaminase, Blood Urea Nitrogen and creatinine by autoanalyzer (Echo).

Histopathological study

Histopathological tests of the liver and kidney were done according to the method described by Farnsworth *et al.*[17]. Organ pieces were fixed in buffer formaldehyde solution (10 %), dehydrated by serial ethanol solution, diaphanized with toluene and embedded with paraffin. Microtome sections were stained with hematoxylin-eosin and examined under a light microscope.

Data analysis

The results are expressed as (mean ± SEM) obtained from n separate experiments. Statistical significance between the groups was analyzed by means of an analysis of variance followed by Tukey-Kramer's multiple comparison tests. P values less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Plants have always been a common source of medicaments, either in the form of traditional preparations or as pure active principles. In many research laboratories, there is therefore much in favour of establishing programs for producing standardized and safe galenical traditional preparations for potential use in primary health care, with the eventual aim of discovering their principles. Even if the active principles have not yet been identified in some of plants used in traditional medicine, the historical evidence of the value of such plants could result in useful preparations, provided they are safe. Evaluation of safety should therefore be prime consideration, even at the expense of establishing efficacy of the preparation[18].

So it was essential to assess the safety of *S. monostachyus*, even if more pharmacological activities have not yet been studied.

Per os administration of Esomo for 28 days did not induce any mortality with doses of 500, 1000, 2000 mg/kg body weight in tested mice. And no signs of adverse effects observable was

detected during the experimental period. Figure 1 shows body weight and organs weights (kidney, liver) of mice at the end of treatment. Esomo administration did not change body, kidney, and liver weights ($p > 0.05$). Food and water intake were also no significantly different in treated-groups compared to control group ($p > 0.05$) (Figure 2).The absence of change in body weight could be related to the lack of variation of food and water intake in treated mice compared to controls. Thus, Esomo extract did not affect the nutritional state of the mice[19].

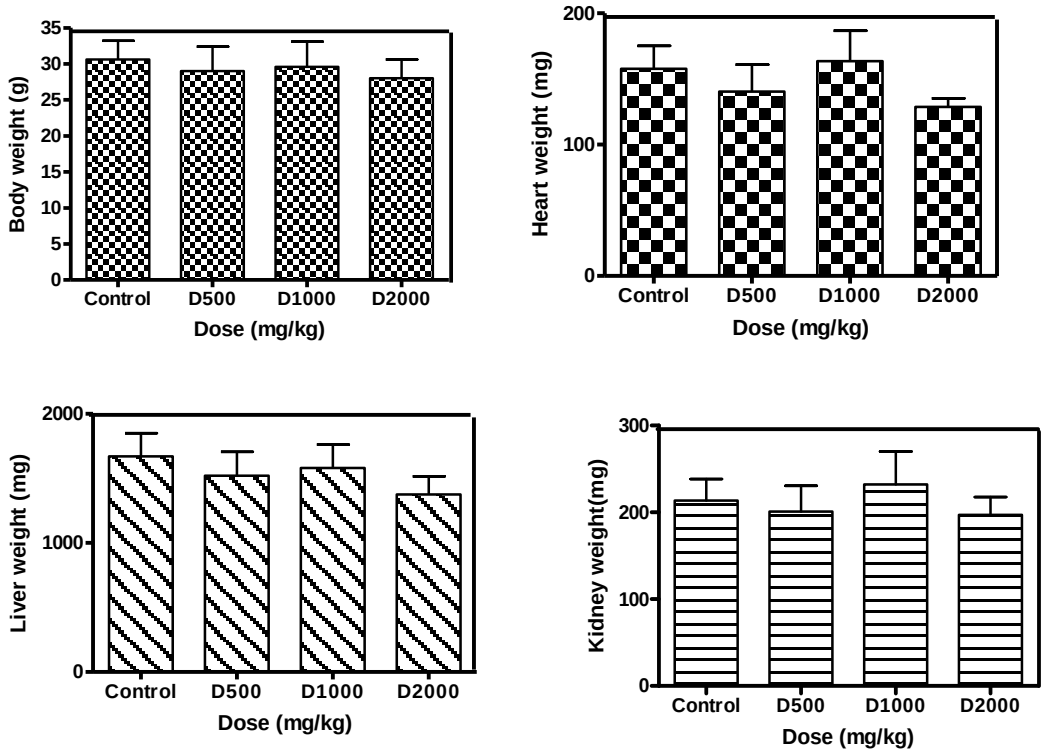

Figure 1: Effect of subacute oral administration of Esomo on body and organ weight of mice. Esomo did not cause any significant changes on body, kidney, and liver weights($p > 0.05$).

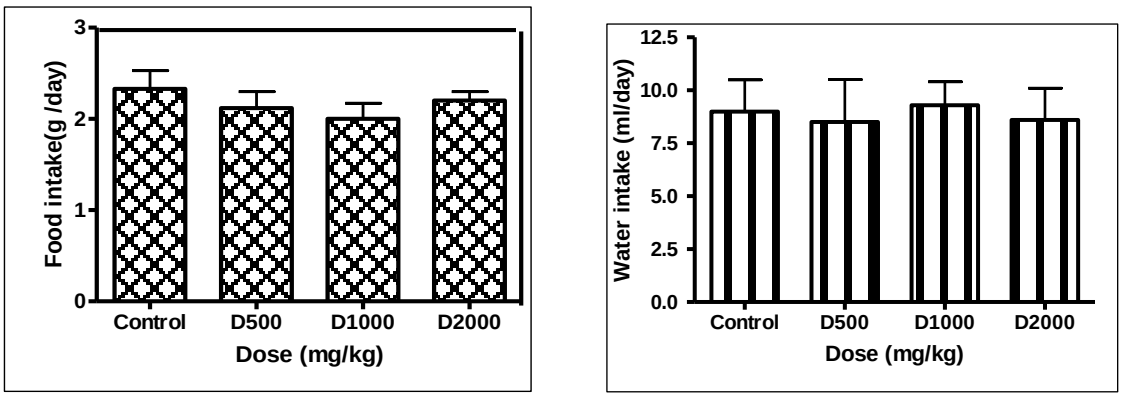

Figure 2: Effect of subacute oral administration of Esomo on food and water intake of mice. Esomo did not cause any significant changes on food and water intake ($p > 0.05$).

Hematological parameters of mice fed with Esomo for 28 days are shown in Figure 3.There was no significant change in erythrocytes number, Leukocytes number, hemoglobin concentration, hematocrit and MCV ($p > 0.05$), while platelets number significantly decreased at 1000 mg/kg b.w. ($p < 0.01$) and 2000 mg/kg b.w. ($p< 0.001$).

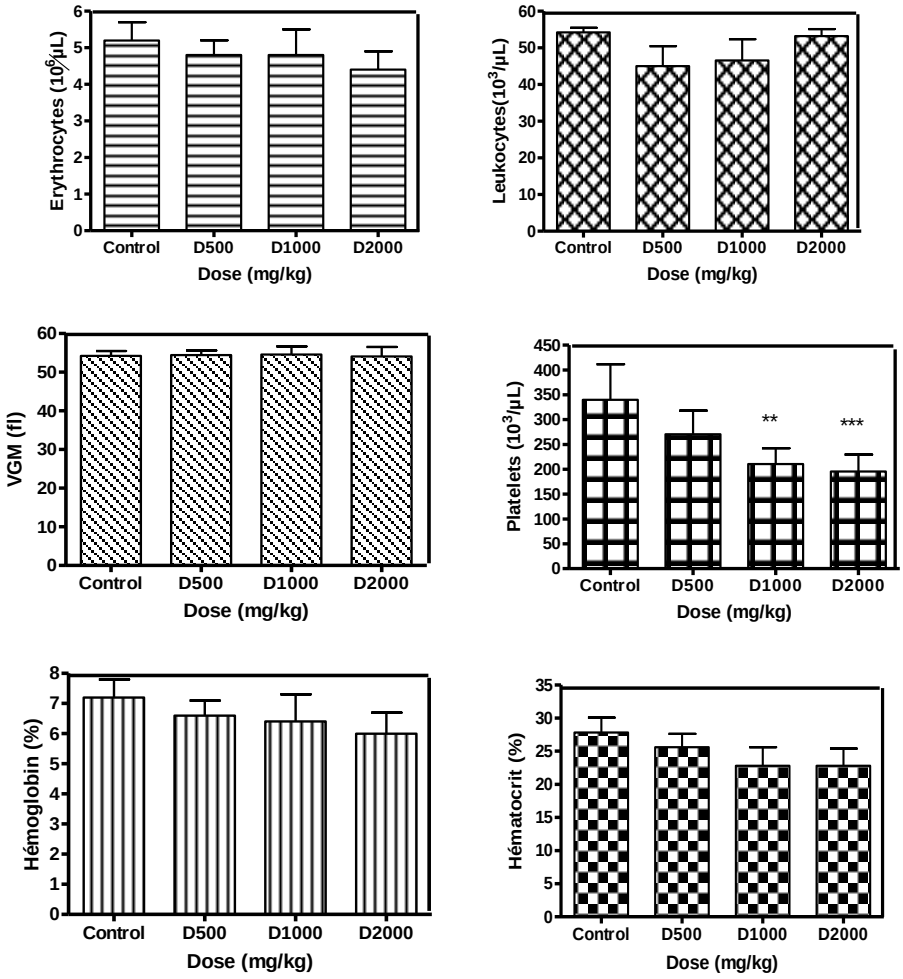

Figure 3: Effect of subacute oral administration of Esomo on erythrocytes number, Leukocytes number, hemoglobin concentration, hematocrit and MCV of mice. There was no significant changes observed in erythrocytes count, leukocytes count, hemoglobin concentration, hematocrit and MCV ($p > 0.05$), while platelets number decreased significantly at 1000 mg/kg b.w. ($p < 0.01$) and 2000 mg/kg b.w. ($p< 0.001$).

Platelets play a fundamental role in hemostasis and are natural source of growth factors. The decrease of platelets is called thrombocytopenia[20].

Many cardiovascular diseases such as arterial hypertension are associated with an increase in blood platelets activity[21-23]. It is well reported in the literature that *Allium sativum*[24] and *Lycopersicum esculenta*[25] may be beneficial in protecting against cardiovascular diseases as a

result of inhibiting platelets aggregation. *S. monostachyus* is a medicinal plant reputed to treat arterial hypertension[6]. Thus, the extract could induce a thrombocytopenia by reducing platelets activity to treat hypertension related with increase in blood platelets activity.

Leukocytes count of mice treated with dose of 500 mg/kg b.w. has showed decrease compared to the control and two others groups. However, it is not statistically significant.

Leukocytopenia is a decrease in a number of leukocytes found in the blood, which places individuals at increased risk of infection. Some anticonvulsant drugs have been associated with a decrease in white blood cell counts[25]. So low white blood cell of this group treated with Esomo, may be due to the presence of anticonvulsant principles in our extract[9].

Biochemical estimations of animal treated with Esomo for 28 days did not affect blood chemical parameters such as Serum Glutamic oxaloacetic Transaminase (AST), Serum Glutamic Pyruvic Transaminase (ALT), Blood Urea Nitrogen (BUN) and Creatinine compared to the control group ($p > 0.05$) (Figure 4).Transaminases (AST and ALT) test are good indicator for hepatocellular injury and creatinine, BUN tests for renal function. Histopathological analysis of mice liver and kidney tissues are shown in Figure 5. Histological features of the liver of control mice showed normal structures (Figure 5_{P1}). Mice treatment with all doses of Esomo did not cause alteration of histoarchitecture of hepatocytes (Figure $5_{P2\text{-}P4}$).

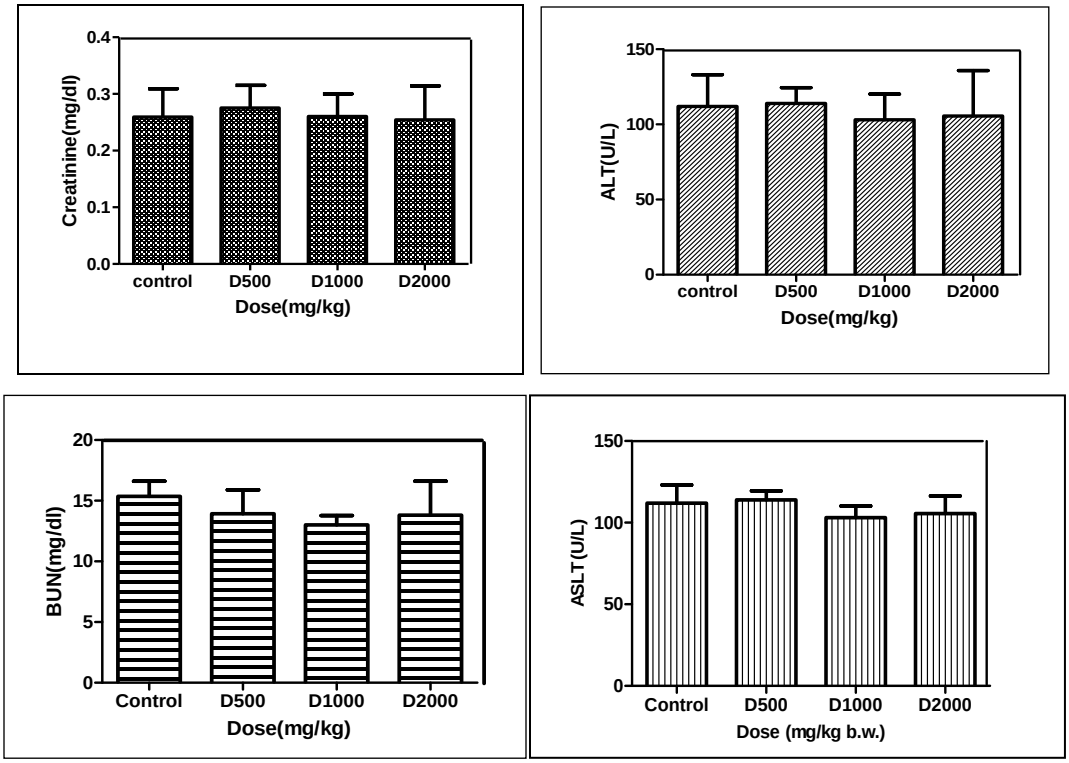

Figure 4: Effect of subacute oral administration of Esomo on biochemical parameters of mice. Esomo did not cause any significant changes on AST, ALT, BUN and Creatinine ($p > 0.05$).

There was no effect on kidneys of mice treatment with a dose of 500 to 2000 mg/kg body weight of Esomo, glomeruli, and tubules appeared normal (Figure $5_{P6\text{-}P8}$) not different from kidney of control mice (Figure 5_{P5}).

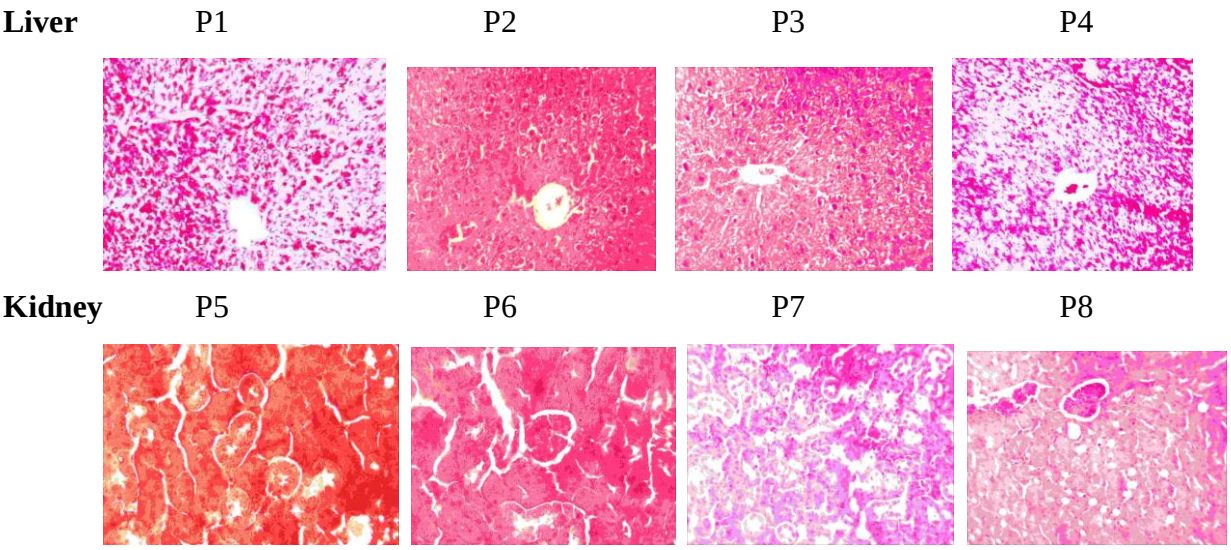

Figure 5: Photomicrographs of subacute oral administration of Esomo on liver and kidney tissue of mice.No histopathological changes were observed. P1: Control, P2: 500mg/kg b.w., P3: 1000mg/kg, P4: 2000mg/kg (Liver). P5: Control, P6: 500mg/kg b.w., P7: 1000mg/kg, P8: 2000mg/kg (Kidney).Scale enlargement: (x 250) for liver and (x 400) for kidney.

Coloration: Hematoxylin-eosin.

All biochemical parameters were normal without any significant difference. So Esomo did not affect liver and renal functions.In addition, histopathological examination of liver and kidney did not reveal signs of toxicity in treated mice. No histopathological changes were observed.

It is well established that free radicals and reactive oxygen species cause cells damage leading to produce many deases[26]. The antioxidant properties of *S. monostachyus* leaves have been demonstrated by several authors[7,27,28]. Thus, Esomo could exert a protective action on hepatocytes, leukocytes, erythrocytes and kidney cells.

CONCLUSION

In this study, hydroethanolic leaves extract of *S. monostachyus* treatment in mice appears to be relatively nontoxic. However, at high doses, our extract induced thrombocytopenia. It is important to note that high doses used in this study are not always correlated to that used in traditional practices. This choice has been done to take preventive action. So the use of Esomomust be done carefully to avoid possible toxic effects of the plant. Further investigations must be conducted to clarify the effects of *S. monostachyus* leaves on platelets aggregation.

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REFERENCES.

1. Awang DVC, Béjar E. Plant Bioassay-The Promise and the Challenge. Journal of Herbs, Species & Medicinal Plants 2001; 8: 1-6.
   DOI: 10.1300/J044v08n01_01
2. Benneth RN, Kidle G, Wallsgrove RM. Biosynthesis of benzylglucosinolate, cyanogenicglucosides and phenylpropanoids in *Carica papaya*. Phytochemistry 1997; 45: 59-66..http://dx.doi.org/10.1016/S0031-9422(96)00787-X
3. Ogbonnia S, Adekunle AA, Bosa MK, Enwuru VN. Evaluation of acute and subacute toxicity of AlstoniacongensisEngler (Apocynaceae) bark and *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) fruits mixtures used in treatment of diabetes. Afr J Biotechnol 2008; 7:701-705.http://www.academicjournals.org/AJB
4. Buisson F, Jaeger P, Lunven P, Pinta M. Food plants of West Africa: botanical, biological and chemical Studies. Leconte, Marseille, France 1965:579 [In French]
   http://www.documentation.ird.fr/hor/fdi:10393
5. Mvé-Mba CE, Menut C, Lamaty G, Zollo PHA, Tchoumbougnang F, Bessière JM. Aromatic plants of tropical central Africa. Part XIX. Volatile components from leaves of two lamiaceae from Cameroon: *Leucas deflexa* Hook. and *Solenostemon monostachyus* (P.Beauv.) Briq. Flav Frag J 1994; 9: 315-317.DOI: 10.1002/ffj.2730090607
6. N'Guessan K, Tiébré M-S, Aké-Assi E, Guédé NZ. Ethnobotanical study of plants used to treat arterial hypertension in traditional medicine, by abbey and Krobou population of Agboville (Cote d'ivoire). Eur J Sci Res 2009; 35: 85-98.http://www.eurojournals.com/ejsr.htm
7. Datté JY, Kpahé F, Offoumou AM. Acute toxicity and antioxidant activity of hydroethanolic extract of *Solenostemon monostachyus* P. Beauv. Leaves. Journal of complementary and integrative medicine 2010; 7: 45.DOI: 10.2202/1553-3840.1492

8. Miyase T, Rüedi P, Eugster CH. Structures of six Coleons (Diterpenoids) from *Solenostemon monostachyus* (P. Beauv.) Briq. (Labiatae). Helvetica ChimicaActa 1980; 63: 95-101. [*In Deutch*]DOI: 10.1002/hlca.19800630110
9. Onu UO. Some pharmacological properties: *Solenostemon monostachyus*. Journal of Herbs, Species and Medicinal Plants 1996;4: 3-7.DOI:10.1300/J044v04n02_02
10. Hodgson E. A textbook of Modern Toxicology. Wiley-Interscience (3rd edition). 2004:672.
11. Kpahé ZF, Konan BA, Datté JY, Offoumou AM (). Action of hydroethanolic leaves extract of *Solenostemon monostachyus* (Lamiaceae) on cardivascular system of mammalian: Blood pressure lowering effects. Int J Pharm Bio Sci 2012; **2**: 310-320. www.ijpbs.com or www.ijpbsonline.com
12. National Research Council. Guide for Care and Use of Laboratory Animal. Eighth Edition, National Academy Press, Washington, 1996.http://grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-Laboratory-animals.pdf
13. Mosihuzzaman M, Choudhary MI. Protocols on safety, efficacy, standardization and documentation of herbal medicine. Pure ApplChem 2008; 80: 2195-2230.Doi:10.1351/pac200880102195
14. OECD. Principles on Good Laboratory Practice: In: handbook, Good Laboratory Practice (GLP), quality practices for regulated non-clinical research and development TDR PRD/GLP/01.2. 2001:226.
15. Waynforth BH, Flecknell P. Injection techniques. In: Experimental and surgical techniques in the rat. Academic Press, London, NY, USA, 1980:30-61.
16. Lamb GM. Manuel of veterinary technics in Kenya published by CIBA GEGY.PP.100. 1987.
17. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. Medicinal plants in therapy. Bull World Health Organ 1985; 63: 965-981. PMID: 3879679 [PubMed - indexed for MEDLINE] PMCID: PMC2536466
18. Pieme CA, Penlap VN, Nkegoun B, Taziebou CL, Tekwu EM , Etoa FX, Ngongang J. Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of *Sennealata* (l) Roxb. (Ceasalpiniaceae). Afr J Biotechnol 2006; 5: 283-289.http://www.academicjournals.org/AJB

19. Maton A. Human Biology and Health. Englewood cliffs, New Jersey, USA Prentice Hall.ISBN 0-13-981176-1. 1993
20. Erne P, Bolli P, Bürgisser E, Bükler FR. Correlation of platelet calcium with blood pressure. Effect of antihypertensive therapy. N Engl J Med 1984; 310: 1084-1088. PMID: 6143260 [PubMed - indexed for MEDLINE]
21. Andrioli G, Ortolani R, Fontana L, Gaino S, Bellavite P, Lechi C, Minuz P, Manzato F, Tridente G, Lechi A. Study of platelet adhesion in patients with uncomplicated hypertension. J Hypertens 1996; 14:1215-1221.PMID: 8906521 [PubMed - indexed for MEDLINE]
22. Mekhfi H, ElHaouari M, Bnouham M, Aziz M, Ziyyat A, Legssyer A. Effects of extracts and tannins from *Arbutus unedo* leaves on rat platelets agregations. Phytother Res 2006; 20:135-139.DOI: 10.1002/ptr.1822
23. Rahman KH, Billington D. Dietary supplementation with aged garlic extract inhibits ADP-induced platelet aggregation in humans. J Nutr 2000; 130: 2662-2665.PMID: 11053504 [PubMed - indexed for MEDLINE]
24. Dutta-Roy AK, Crosbie L, Gordon MJ. Effect of tomato extract on human platelets aggregation *in vitro*. Platelets 2001; 12: 218-227.PMID: 11454256 [PubMed - indexed for MEDLINE]
25. Nicholson RJ, Kelly KP, Grant IC. Drug points: Leucopenia associated with lamotrigine. BMJ 1995; 310: 504. Doi: http://dx.doi.org/10.1136/bmj.310.6978.504b ; PMCID: PMC2548879
26. Halliwell B. Antioxidant and human diseases: A general introduction. Nutr Rev 1997; 55: S44-S49.DOI: 10.1111/j.1753-4887.1997.tb06100.x
27. N'Guessan HA, Dago DCE, Mamyrbekova-Bekro JA, Békro YA. CCM of selective extracts of 10 plants used in the traditional treatment of hypertension in Côte d'Ivoire. Eur J Sci Res 2011; 66: 575-585. [In French]http://www.europeanjournalofscientificresearch.com
28. Okoko T, Ere D. Antioxidant activities of *Solenostemon monostachyus* leaf extract using in vitro methods. Sci Res Essays 2012; 7: 621-626.DOI: 10.5897/SRE10.743