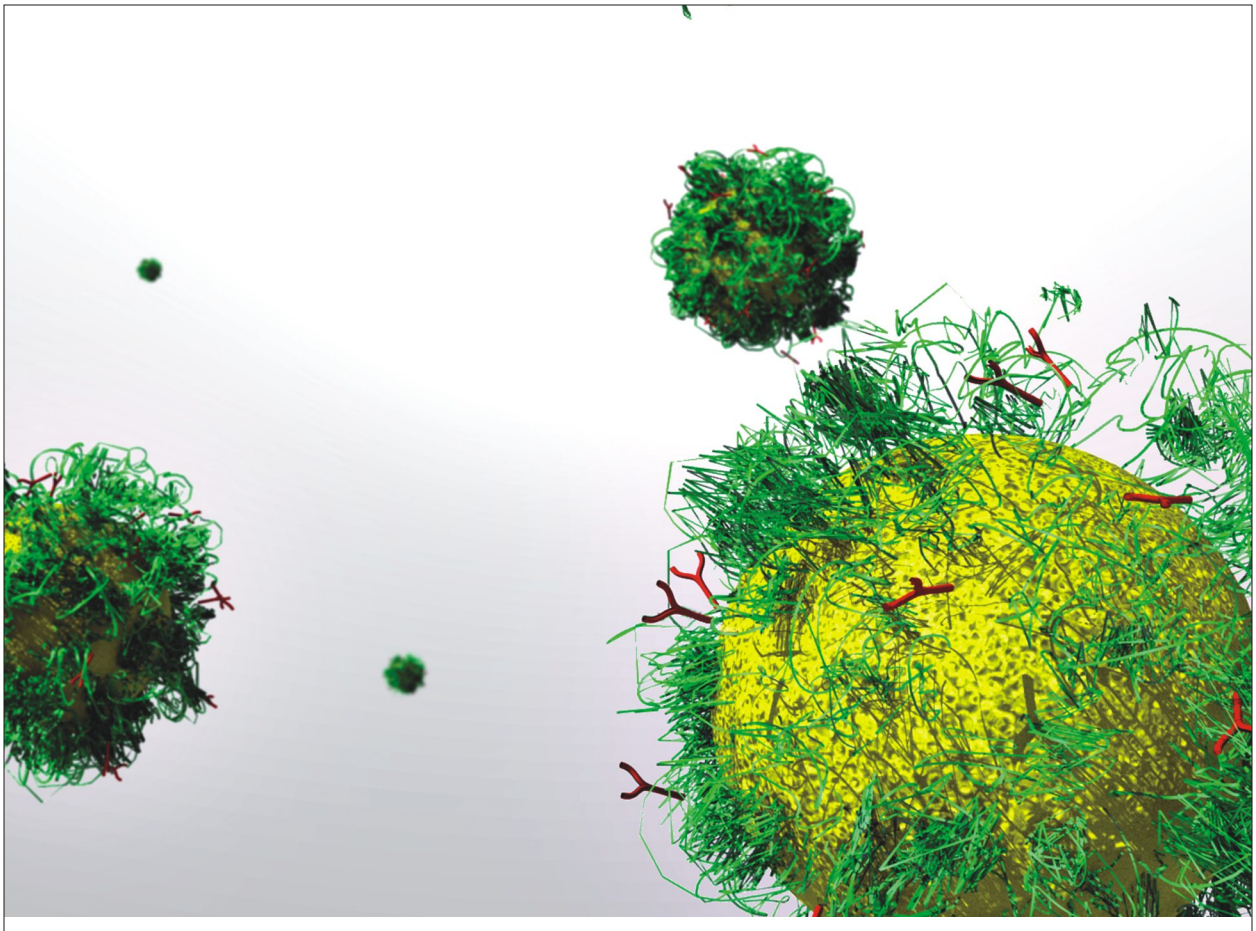

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Safety and Standardization Indices on Selected Anti-malarial Herbs: Effect on Haematology and Serum Enzyme Levels

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ABSTRACT

This study was designed to evaluate the effect of ethanolic stem bark extract of *Nauclea latifolia*, *Cylicodiscus gabunensis* and *Araliopsis souyauxii*; three plants used locally as anti-malarials, on haematology and serum enzyme levels in albino rats. Employing a 3×3 factorial arrangement, thirty six male albino rats were randomly assigned into nine groups of four rats each, treated daily with ethanol extract corresponding to 0, 125 and 225 mg kg^{-1} b.wt. of the three plants. Treatment was administered orally for two months, after which the animals were sacrificed for estimation of study parameters. All data were subjected to analysis of variance, with conclusions drawn at 5% probability level. Results obtained showed that while *N. latifolia* and *A. souyauxii* gave no evidence of potential haemo-toxicity, *C. gabunensis* at 225 mg kg^{-1} b.wt. significantly reduced haemoglobin content and packed cell volume of the animals. Although alanine transaminase revealed no significant differences, all plants induced significant elevation in alkaline phosphatase levels at both 125 and 225 mg kg^{-1} b.wt. Aspartate transaminase was also significantly elevated in all plant groups at 225 mg kg^{-1} b.wt. Overall, the potential toxic effect of these anti-malarial herbs was observed to be more on serum enzyme levels, than on haematology. In quest for standardized anti-malarial preparations from these herbs, we recommend a dose no more than 225 mg kg^{-1} b.wt. for *N. latifolia* and *A. souyauxii* and 125 mg kg^{-1} b.wt. for *C. gabunensis*.

Key words: Anti-malarial herbs, haematology, serum enzymes, dosage safety, rat

INTRODUCTION

Malaria remains a major disease afflicting millions of people yearly in tropical and subtropical parts of the globe; with an estimated one million child deaths each year in Sub-Saharan Africa, due to the disease (Anon, 2007). Local peoples in several African communities rely on herbal preparations for treatment of malaria as well as other common health maladies (Akah *et al*., 1998; Adebayo and Krettli, 2011; Kolawole *et al*., 2011). On a global scale, the utilization of plant based preparations in therapeutics is currently gaining increased attention in the intellectual and public domains. This is evidenced in the huge volume of research publications in this regard, as well as the formal recognition of phyto-therapy by governments and proponents of orthodox medicine (Adeshina, 1998; Adeniyi *et al*., 2010; Ekaluo *et al*., 2013). This is due partly to the realization of the immense health benefits that can accrue due to the proper utilization of locally available plant bio-resources (Brisibe *et al*., 2008).

Nauclea latifolia (Smith), commonly known as "African peach" is an evergreen shrub that is widespread in humid tropical rainforest zones and grows up to 200 m. It is popular with local peoples, as infusions and decoctions of the bark and leaves are used for the treatment of stomach pains, fever, diarrhoea, malaria, amongst others (Benoit-Vical *et al*., 1998; Okiemy-Andissa *et al*., 2004). *Cylicodiscus gabunensis* (Harms) commonly known as "African green heart" is a large tree with a cylindrical trunk, widespread branches and a bark that has a strong odour. Its known medicinal use include; preparation of the stem bark for the treatment of headache, rheumatism, gastro intestinal disorders, vomiting, venereal diseases, malaria and psoriasis (Adjanohoun *et al*., 1996). *Araliopsis soyauxii* (Engl) is a large tree of that grows up to 25 m tall, has compound leaves about 5-7 in number, unisexual flowers and four-seeded fruits. It is used to treat gonorrhea and malaria; and the twigs and small branches, even though bitter, are widely used as chewing sticks in the rural areas of Nigeria and Cameroon (Menut *et al*., 1994).

Despite their availability, affordability and popularity with local peoples, the vast majority of herbal formulations/decoctions though, are known to be utilized with little or no form of safety evaluation/standardization (Tiwari *et al*., 2004). As knowledge of phyto-chemistry and phyto-toxicology of plant species remain relevant in the quest for safe and efficient utilization of herbal remedies, evaluating the effect of herbs on heamatological and biochemical parameters fits nicely on a phyto-safety backdrop; as it represents one way of assessing the toxicological potential of plant based therapies (Akpanabiatu *et al*., 2005; Shafaei *et al*., 2011). This is the underlying rationale of this study, especially as *Nauclea latifolia, Cylicodiscus gabunensis* and *Araliopsis souyauxii* are among the popular herbs utilized in anti-malarial preparations, in parts of Cross River and other states of Southeastern Nigeria (Arise *et al*., 2012; Ikpeme *et al*., 2013).

MATERIALS AND METHODS

Collection and preparation of plant materials: Authenticated stem barks of *N. latifolia, C. gabunensis* and *A. souyauxii* were obtained from the herbarium unit of Department of Forestry, University of Calabar, Calabar. They were oven dried at 80°C and ground into powder using a heavy duty blender (Christison 37 BLIB, model 240c BC). Soxhlet extraction using 98% ethanol was carried out and the extract was collected in a round bottom flask. The ethanol and water were removed using rotary evaporator (Buchi RE 111) under reduced pressure at 60°C. The resulting extract paste was then stored in the refridgerator until needed.

Experimental design and animal treatment regimen: Thirty six male albino rats of approximately two months of age were obtained from the Animal House of Department of Zoology and Environmental Biology, University of Calabar, Calabar. The animals were housed in standard wire mesh cages, with light period of 12 h day^{-1}, temperature of 27±2°C and allowed free access to feed and water. In a 3×3 factorial layout, the animals were randomly assigned into nine groups of four rats each, treated with ethanol extract corresponding to 0, 125 and 225 mg kg^{-1} b.wt. of *N. latifolia, C. gabunensis* and *A. souyauxii*. One week of acclimatization and quarantining was allowed before commencement of treatment and all rats were handled in accordance with the standard guide for the care and use of laboratory animals, as laid down in the EU directive 2010/63/EU for animal experiments and mandated by the Animal Research Committee of the Department of Genetics and Biotechnology, University of Calabar, Calabar.

Animals were treated daily with the stated doses of plant extract via oral gavage for a period of two months. The body weight (kg) of the animals in each group were determined (ScoutPro SPU601, Ohaus) once every week and this was used to calculate the amount of extract that was administered, as shown in Eq. 1:

$$\text{X mg of extract} = \text{Group dose} \times \text{kg body weight of animals} \quad (1)$$

Twenty four hours after the last treatment round, the animals were sacrificed under chloroform anaesthesia.

Determination of haematological parameters: Blood was collected via cardiac puncture into K_3EDTA tubes and haemoglobin content (g dL^{-1}); red blood cell count (10^9 L^{-1}); white blood cell count (10^9 L^{-1}); platelets count (10^9 L^{-1}) and packed cell volume (%), were determined using standard procedures (Schalm *et al.*, 1975; Dacie and Lewis, 1991).

Determination of serum enzyme levels: Blood was collected in plain tubes, allowed to clot and spun at 2500 rpm for 5 min in a centrifuge (Wisperfuge 1384, Tamson, Holland). Serum was collected into labeled plain tubes and used for estimation of enzyme levels. The methods described by the "International Federation of Clinical Chemistry" were used to determine levels of Aspartate Transaminase (AST) (Bergmeyer *et al.*, 1986a) and Alanine Transaminase (ALT) (Bergmeyer *et al.*, 1986b), using a Spectrophotometer (Jenway 6405, Essex, England) while Alkaline Phosphatase (ALP) levels were determined using p-nitrophenylphosphate according to the method of Bessey *et al.* (1946).

Statistical analysis: All data generated was subjected to Analysis of Variance (ANOVA) to check for significant differences between the treatment groups at 5% probability level and results were expressed as Mean±SEM.

RESULTS AND DISCUSSION

The clinical significance of haematological and serum biochemical parameters; as physiological indicators of stress in animals, is well established (Hymavathi and Rao, 2000; Guyton and Hall, 2006; Ambali *et al.*, 2010; Ibiang *et al.*, 2013). Increase in serum levels of AST, ALT and ALP may indicate hepatotoxicity while haematological parameters such as RBC, Hb content and PCV, when reduced beyond normal levels also indicate toxicity of plants, drugs, chemicals and other xenobiotics in the animal system (Ambali *et al.*, 2010).

Treatment with extract of *C. gabunensis* at 225 mg kg^{-1} b.wt. resulted in significantly ($p<0.05$) lower haemoglobin content in the animals (Table 1), an indication of possible adverse effect on blood oxygen carrying capacity (Raven *et al.*, 2005). Red blood cell count was observed to be significantly elevated due to *N. latifolia* treatment at 225 mg kg^{-1} b.wt. *A. souyauxii* administered at 125 mg kg^{-1} b.wt. also led to significantly increased RBC. It appears then that at the above mentioned doses, both these plants possess no anti-haematinic properties. In contrast, they improved RBC count, perhaps via a stimulation of erythropoietin release in the kidney; as this organ is a humoral regulator of RBC production (Polenakovic and Sikole, 1996; Sanchez-Elsner *et al.*, 2004). Significantly reduced white blood cell count were observed due to *N. latifolia* and *C. gabunensis* treatments at both 125 and 225 mg kg^{-1} b.wt. as compared with the control (0 mg kg^{-1} b.wt.). In *A. souyauxii* treated groups, a dose dependent reduction was observed,

Table 1: Haematological parameters and serum enzymes of rats after treatment with ethanol extract of selected anti-malarial herbs

	N. latifolia (mg kg^{-1})			*C. gabunensis* (mg kg^{-1})			*A. soyauxii* (mg kg^{-1})		
Parameters	0	125	225	0	125	225	0	125	225
Hb (g dL^{-1})	13.32^{b}±0.67	12.60^{b}±0.41	13.25^{b}±0.48	12.5^{b}±0.29	12.30^{b}±0.41	10.00^{a}±0.41	13.12^{b}±0.58	13.51^{b}±0.65	12.59^{a}±0.96
RBC (10^{12} L^{-1})	7.28^{a}±0.03	7.32^{a}±0.01	8.08^{b}±0.01	7.20^{a}±0.01	8.07^{b}±0.02	7.01^{a}±0.00	7.19^{a}±0.01	7.9^{b}±0.010	7.25^{a}±0.01
WBC (10^{9} L^{-1})	5.15^{c}±0.01	4.53^{b}±0.04	4.76^{b}±0.04	5.15^{c}±0.02	3.95^{a}±0.04	4.51^{b}±0.04	5.17^{c}±0.05	4.90^{c}±0.04	3.88^{a}±0.04
PLT (10^{9} L^{-1})	205.0^{a}±0.04	252.5^{c}±2.180	285.0^{e}±0.410	207.5^{a}±0.29	274.7^{d}±0.410	230.1^{b}±0.410	210.5^{a}±0.5	289.3^{e}±0.410	276.9^{d}±0.410
PCV (%)	39.40^{c}±0.41	36.75^{b}±0.48	39.5^{c}±0.650	40.5^{c}±0.960	36.20^{b}±0.82	33.0^{a}±0.820	38.75^{c}±0.25	39.5^{c}±0.290	36.05^{b}±1.29
AST (iu L^{-1})	120.1^{a}±0.07	122.0^{a}±0.480	125.5^{b}±0.290	121.6^{a}±0.41	120.2^{a}±0.250	126.0^{b}±0.410	122.8^{a}±0.63	125.7^{b}±0.250	127.2^{c}±0.250
ALP (iu L^{-1})	132.1^{a}±0.41	235.6^{c}±0.410	352.3^{e}±0.480	131.7^{a}±0.48	255.4^{d}±0.410	261.8^{e}±0.410	135.7^{a}±0.43	262.6^{d}±0.410	189.2^{b}±0.410
ALT (iu L^{-1})	85.5^{a}±0.290	86.8^{a}±0.410	88.0^{a}±0.710	85.7^{a}±0.430	86.9^{a}±0.630	86.2^{a}±0.430	86.5^{a}±0.960	86.0^{a}±0.500	85.2^{a}±0.250

Values are Mean ±SE. Values across the table with similar superscript are not significantly different at 5% based on ANOVA

with 225 mg kg^{-1} b.wt. group having significantly lower WBC than both control and 125 mg kg^{-1} b.wt. Being the cellular defensive component of the animal blood system, elevated WBC is usually interpreted to mean a response to invasion by a biological or chemical entity; while a reduction in this parameter, as seen on treatment with these plant extracts, highlights possible diminished production, or redistribution from blood to other body tissues (DeBaun, 2005). Significant dose dependent increases were observed in platelets count due to *N. latifolia* treatment at doses of 125 and 225 mg kg^{-1} b.wt. *C. gabunensis* and *A. souyauxii* also significantly elevated platelets, but this was not in a dose dependent manner. Elevated platelets levels by these plants could indicate a stimulatory effect on thrombopoietin (Li *et al*., 1999), implying a capacity to militate against thrombocytopenia (Patil *et al*., 2013). Packed cell volume is a measure of total haematocrit levels (Dacie and Lewis, 1991) and its reduction upon treatment with a substance might be indicative of haemo-toxicity. Significant dose dependent reduction in PCV was observed due to treatment with *C. gabunensis* with the lowest value observed in the 225 mg kg^{-1} b.wt. group. This group also had the lowest RBC count, although it was not significantly different from control. Given the significant reduction in PCV due to *C. gabunensis* at 225 mg kg^{-1} b.wt. a significant reduction in RBC in this group would have been expected-but this was not the case (Table 1). Mean PCV of control albino rats have been reported to be 45.20% (Abdel Aziz *et al*., 2010); 44.21% (Savithri *et al*., 2010); 38.51% (Adjroud, 2009); 21.20% (Oyewole *et al*., 2009); and 32.50% (Kolawole and Alemika, 1996). The value (33.0%) observed in *C. gabunensis* group of 225 mg kg^{-1} b.wt. then, might be taken as not (far) below the normal range. Nevertheless, of the three plants as used in this study, only *C. gabunensis* at 225 mg kg^{-1} b.wt. exhibited slight haemo-toxicologic potential, taking into cognizance the significant lower PCV and Hb values observed in this study.

While Alanine Transaminase (ALT) showed no significant ($p>0.05$) differences between the treatment groups, the reverse was the case for Aspartate Transferase (AST) which revealed significantly elevated levels at 225 mg kg^{-1} b.wt. in all three plant groups. In addition to the observed significant increases in Alkaline Phosphatase (ALP) due to respective plant extracts at all doses higher than 0 mg kg^{-1} b.wt. this perhaps indicates that much of the potential toxic effect of the herbal preparations as used in this study is on serum biochemical parameters, rather than on haematology. This is in line with the recent findings of Arise *et al*. (2012) in their studies with aqueous extracts of *N. latifolia*. In the light of the significance of serum enzyme levels as indicators of hepatotoxicity, elevated levels of AST and ALP elicited by the administration of ethanolic anti-malarial herbal preparations in this study is notable and lends impetus to calls for safety evaluation/standardization of (local) herbal therapies.

Phytochemicals in plants have been known to be responsible for various physiological and biochemical effects in animals (Wang *et al*., 2004). And our previous studies, as well as those of other authors reveal that the above plants have a rich spectrum of these (Tchivounda *et al*., 1991; Karou *et al*., 2011; Tene *et al*., 2011; Ikpeme *et al*., 2013). Complex interactions between these bio-active components could result, perhaps to a large extent, in the potential toxicological effect of these anti-malarial plants observed in this study.

CONCLUSION

In contrast with serum enzyme levels, the changes observed in haematological indices in this study appear rather slight; except for *C. gabunensis* at 225 mg kg^{-1} b.wt. which resulted in a significantly reduced packed cell volume and haemoglobin content. But in as much as the safe and efficient utilization of phyto-preparations continue to remain a relevant issue; especially with regards to treatment of a disease of endemic status as malaria, we conclude that the three plants have shown a notable potential to negatively affect serum levels of AST and ALP, at doses up to 225 mg kg^{-1} b.wt. Should standardized anti-malarial preparations be required from *Nauclea latifolia* and *Araliopsis souyauxii*, we recommend simply the administration at doses less or no more than 225 mg kg^{-1} b.wt. especially where this is found to be efficacious. For *Cylicodiscus gabunensis*, a dose of 125 mg kg^{-1} b.wt. is recommended as it showed no haemo-toxicity in the rats.

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