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ASSESSMENT OF SERUM ELECTROLYTES IN RABBITS TREATED WITH AQUEOUS EXTRACTS FROM *PHYLLANTHUS AMARUS* (Euphorbiaceae)

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ABSTRACT
In the presence of medicinal plants, the activity of the electrolyte in cells would affect the body. In this experimental study , which lasted four weeks of treatment and one week without treatment, *in vivo* assessment of serum electrolytes (Ca^{2+} , K^+ , Na^+ and Cl^-) rabbits treated with an aqueous extract from *Phyllanthus amarus* were determined . After rabbits being treated intra peritoneally with various doses ranging from 0 to 100 mg/kg of body weight, sera obtained were compared with those of control subjects (normal values). Following this, the results show statistically significant changes for the effect of doses of the injected product with Ca^{2+} and the effect of product exposure time with Na^+ and K^+. These different manifestations of electrolytes in the presence of aqueous extracts from *Phyllanthus amarus* would affect the activity of cells with effects on vital organs such as the heart, the kidney and the liver. This could confirm the effects of aqueous extracts from *Phyllanthus amarus* in some treatments such as diabetes and high blood pressure.

KEYWORDS
Phyllanthus amarus, Electrolytes and Rabbits.

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INTRODUCTION
The impressive richness of Africa's flora, justifies the use of plants for therapeutic purposes by 70 % of all these people. The identification of plants in 1979 enumerated the existence of more than 5 000 plant species with medicinal properties[1]. 761 species of medicinal plants and 1 421 medicinal recipes have been identified in Côte d'Ivoire[2]. Indeed, ethnobotanists and chemists involved in these programs are more committed to have modern medicine profited from it[3]. The use of these plants in

the population is related to traditional customs, to the richness of the flora, the purchasing power of the interested and availability of natural products. These natural substances that have biological activities could be potentially therapeutically useful[4].

However, the improper use of plant extracts could be associated with the problems of dose administrations which are at the origin of many renal diseases in Africa in general and in Côte d'Ivoire particular with 10.45% of poisonings by medicinal plants[5, 6].

Among the plants of the African pharmacopoeia , the aqueous extracts from *Phyllanthus amarus* , a Euphorbiaceae (Shunt and Thom) have been explored the serum parameters, namely at the level of kidneys or prolonged use could possibly cause a malfunction of that organ on the one hand; and on the other hand would probably be cardioprotective[7,8].

The action of the aqueous plant extracts on different organs could only be done if reassessment of electrolytes at the cellular level is satisfactorily explained on the scientific level.

This study on reassessment of serum electrolytes in rabbits treated with aqueous extracts from *Phyllanthus amarus* allows thinking on the possible mechanisms existing at the cellular level and which have an impact on the overall condition of an organism. In addition, it is important to remember that the natural product extract contains 24.05 % saponins, 17.50 % tannins, 5.47% oxalates, 2.56 % alkaloids and cyanogenic glycosides[9]. Besides, this plant has, pharmacologically, antiviral[10, 11, 12, 13], anti- hepatotoxic[14, 15] and anti- hyperglycemic properties[16, 17, 18].

Works on the action of serum electrolytes such as calcium (Ca^{2+}), chlorine (Cl^-), potassium (K^+) and sodium (Na^+) have been mentioned by many authors[19, 20, 21, 22, 23, 24, 25] and have shown their prevailing role on both cells and body rebalancing.

MATERIAL AND METHODS

The biological material was made of rabbit from farms in the area of Bingerville (Côte d'Ivoire). They are from the *Néozealander* and *Cunistar* crossbreeding. For this study, thirty (30) rabbits (15 males and 15 females) aged of two (2) months were selected.

In addition, the laboratory equipment used for the assay of the various parameters was formed by a Jouan centrifuge, a flame spectrophotometer SEAC *fp* 20, a HITACHI 704 UV-visible spectro photometer, a set of reference assay kits (BIOMERIFUX ®, BIOSYSTEME® and BIOLABO®).

In this study, the animals used had a body weight of about one (1) kilogram. They were divided into batches of three (3) rabbits (male or female) per cage, acclimated for two (2) weeks and fed daily with 150 g of granules used as nutrient maintenance.

Before any treatment corresponding to the first week of study (W_1), single blood samples at the marginal ear vein were made in the morning, between six and eight hours on each animal, subjected to fasting for about ten hours in order to collect the basic physiological parameters (controls). Following these samples, injections of aqueous extracts from various concentrations of 0; 5; 10; 50 and 100 mg/kg of body weight were performed on animals identified for this purpose. These operations were carried out during the next four weeks corresponding to W_2, W_3, W_4 and W_5. One week after cessation of treatment (W_6), samples were carried out on animals. These blood samples were centrifuged at 3000 rpm during ten minutes. Sera obtained after settling were then frozen to -20 °C and used to determine the values of the various parameters such as ionic calcium, sodium, potassium and chlorine.

Determination of various ions identified was made according to specific principles for each element. In the case of calcium, it forms in alkaline environment, a purple complex with complex O-cresol phthalein whose intensity is measured to a wavelength λ that equals to 570 nm UV-visible spectrophotometer while chloride ions with mercuric thiocyanate cause the release of thiocyanate ions which form with ferric nitrate in the presence of nitric acid, a red complex whose intensity is measured by absorbance at a wavelength λ that equals 450 nm UV-visible spectrophotometer.

Regarding sodium and potassium, determination is done in the presence of a source of high constant heat flux to about 2000° Kelvin. At this temperature, sodium and potassium ions will be excited and emit respectively to different wavelengths λ equals to 589 nm and at 767 nm of individual radiations whose intensities are converted into electrical signals, measured in a SEAC *fp* 20 flame spectrophotometer[26].

The analysis methods used in operating results included the average values, the standard deviation for each batch of rabbits collected during the experiment and the calculation of percentage change of average determined by the following formula:

$$\% \text{ of change on average} = \frac{X_i - X_0}{X_0} \times 100$$

X_0: Parameter values for control rabbits before the first week of treatment.

Xi: Parameter values for rabbits treated with different concentrations of products during the experimental period.

Moreover, the statistical test for different parameters obtained was performed by analysis of variance with two (2) factors whose method has been described by many authors[27, 28 29]. The statistical test was adapted by computer software of statistical analysis called STATVIEW®. The statistical test for poses for each parameter analysis during the experiment "H" poses the following assumptions:

H_1: there is no significant difference between the doses of the product injected into rabbits

H_2: There is no significant difference over time for doses of the products injected into rabbits

In the event the assumptions would be rejected with a precision a equaled to 0.05 after comparison of observed factors (F_{obs}) and theoretical factors (F_{th}), the meaning of the F test would result in a decomposition chart[30]. These statistical tests allowed identifying both dose effects factors and product exposure time in different serum electrolytes firstly and secondly to assess their actions both at the cellular and affected organs levels during treatment about 'possible variations in Ca^{2+}, K^+, Na^+ and Cl^- parameters.

in the presence of aqueous extracts from *Phyllanthus amarus*.

RESULTS AND DISCUSSION

This work on the assessment of serum electrolytes in rabbits treated with aqueous extracts from *Phylanthus amarus* yielded average values of calcium serum levels, chlorine, potassium and sodium that are presented in Table No.1 corresponding to data in untreated and identified animals as witnesses[21]. The Table No.1 shows the averages and standard deviations obtained during week W_1 values for the various parameters which are calcium 94 ± 4.43 mg/1, sodium 141.893.96 mEq/1, potassium 3.89 ± 0.37 mEq/1 and chlorine 100.85 mEq/1.

The Table No.2 corresponding to data in treated animals is within the four weeks from W_2 to W_5 and one week after stopping treatment W_6. Thus W_2, 100 % of K^+ values between 4.38 ± 0.38 mEq/1 and 5.22 ± 0.25 mEq/1 are out of the reference data. The same observation was also made with W_3 between Na^+ values between 148.75 ± 2.06 mEq/1 and 142.00 mEq/1 and that of K^+ between 5.40 ± 0.35 mEq/1 and 4.42 ± 0.68 mEq/1. In addition to week W_4, only 60 % of K^+ values are higher than referenced values while the data obtained during Week W_5 and after stopping treatment W_6 show that 80% of K^+ values are increased compared to historical control subjects.

Following these data, statistical analyses were used to identify significant summary parameters grouped in Table No.3 corresponding to the decomposition analysis of electrolyte variances with an accuracy of 5%. Table No.3 shows that the aqueous extract from *Phyllanthus amarus* has a dose effect expressed on Ca^{2+} while time exposure effect is expressed on Na^+ and K^+. But on the other hand, any of these two effects was expressed on Cl^-.

Changes shown in Figures No.1, 2 and 3 relating to the reassessment of serum electrolytes in treated rabbits with different doses of aqueous extracts from *Phyllanthus amarus* over time allowed for a discuss We notice while observing calcium data in Table No.2 and No.3 that statistical analyses show significant effects of the injected product doses produced during the time on Ca2+ ($p < 0.05$). This information is confirmed by the appearance of Figure No.1, which shows an increase during the fourth week of treatment (W_4). These variations in the data appear to be related to the different doses of the products used. In addition, the lowest percentage changes during treatment were obtained with the doses of 10 mg/kg of body weight. This allows showing that the use of different doses of *Phyllanthus amarus* over time could cause an increase in the calcium serum level. Compared to previous data[25] the increase in calcium serum could be explained either by a release of calcium from the extracellular medium from the endoplasmic reticulum or by inhibition of membrane calcium channels. Considering other studies[19, 20], this increase in calcium serum may lead to metabolic disturbances with damage to kidneys, thyroids, bones, and also the reduction of vitamin D. This information allows us to consider the aqueous extracts from *Phyllanthus amarus* as a natural substance which is probably involved in calcium regulation mechanism.

Regarding sodium, statistical analyses showed that in Table No.3, there is a significant effect of exposure time of the injected product into the serum parameter. In addition, observation of Table No.2 and the general appearance of Figure No.2 where the different doses during treatment show on histograms a tendency to increase for the first two weeks and tend to reduce for the last two weeks. In this Figure No.2, the lowest values were obtained for doses of 10 mg/kg of body weight.

These data evidence show that the use of different doses of *Phyllanthus amarus* over time should cause disturbances in sodium regulation. This dysfunction is involved in the renal and hormonal regulation (retina, angiotensin and aldosterone) and possibly in energy deficit at the membrane level. During the first treatment periods, the trend to increase in sodium serum could explain its release in the cell after the mechanism of passive diffusion in the absence of a permanent mechanism for regulating sodium at the membrane level. These findings are in agreement with previous data [22, 2, 24]. Compared to previous work[19, 20], increasing values of sodium serum should evoke an attack from various sources particularly at the levels of dehydration, excess of salt or hypothalamic irritation.

As for potassium, statistical analyses show that with the Table No.3, there is a significant effect of exposure time of the product on the report parameter to control subject ($p < 0.05$). In addition, observation of Table No.2 and the general appearance of Figure No.3, the histograms show a reduction in the percentage changes of potassium during treatment weeks W3 and W4 with different doses of injected products. These data show that the use of *Phyllanthus amarus* over time such as sodium should probably cause disturbances in the regulation of potassium with certainly a hormonal involvement such as aldosterone. This would explain during the first treatment periods, the trend of reduction in potassium serum with its penetration in the cell after the mechanism of passive diffusion for lack of potassium regulation at the membrane level. This could be in agreement with some authors' data[22, 23, 24]. Taking into account previous data[19, 20], the reduction in potassium serum values imply various effects with either at the renal and heart levels or in muscle, accompanied by significant metabolic disorders.

Regarding chlorine, statistical analyses show with table No.3 that there is no significant effect of the treatment on chlorine compared to the control subject ($p<0.05$). This information would indicate that the use of different doses of aqueous extract from *Phyllanthus amarus* over time should not cause physiological disruption of chlorine at both cell and organs levels.

Table No.1: Mean serum values of animal electrolytes before treatment (W_1) with N=30

Parameters	Averages	Minimal	Maximum	Standard deviation
Ca^{2+} (mg/l)	94	78	98	4.43
Na^{+} (mEq /l)	141.89	135	149	3.96
K^{+} (mEq/l)	3.89	3.39	4.70	0.37
Cl^{-} (mEq/l)	100.85	96	107	3.04

BN: N corresponds in keeping with the sample

Table No.2: Mean serum values of animal electrolytes during treatment

		Doses of the products injected to rabbits (mg/kg of body weight)					
		0	5	10	50	100	
		Dose identification code					
weeks	Setting	C_0	C_5	C_{10}	C_{50}	C_{100}	% OVR
W_2	Ca^{2+} (mg/l)	94.25±2.63	93.5±1.39	92.4±4.93	91.2±7.88	92.6±2.30	0
	Na^{+} (mEq /l)	142.25±3.30	142.00±4.32	142.82±7.03	145.00±4.2	141.5±5.89	0
	K^{+} (mEq/l)	4.38±0.38	4.6±0.71	5.22±0.25	4.95±0.74	4.82±1.04	100
	Cl^{-} (mEq/l)	102.5±2.64	102.33±2.87	102.83±5.15	104.00±3.8	102.5±3.27	20
W_3	Ca^{2+} (mg/l)	95.00±1.15	91.66±4.27	92.33±5.82	95.80±1.47	91.80±5.31	0
	Na^{+} (mEq /l)	148.75±2.06	144.83±6.76	149.50±8.14	144.50±3.62	142.00±5.47	100
	K^{+} (mEq/l)	5.28±0.26	4.80±0.79	5.40±0.35	4.58±0.72	4.42±0.68	100
	Cl^{-} (mEq/l)	105.50±1.73	103.50±4.84	106.6±6.37	103.30±2.08	110.60±22.42	60

W_4	Ca^{2+} (mg/l)	95.00±4.35	96.00±1.09	94.40±2.57	96.83±1.16	97.16±0.75	0
	Na^+ (mEq /l)	141.50±2.64	140.80±4.16	138.30±4.45	138.50±3.20	142.83±3.81	0
	K^+ (mEq/l)	5.30±0.80	4.18±0.42	3.93±0.68	4.96±1.06	4.78±0.81	60
	Cl^- (mEq/l)	102.25±1.50	100.83±2.99	99.66±3.19	99.66±2.50	102.50±2.34	0
W_5	Ca^{2+} (mg/l)	94.00±2.64	95.33±3.01	93.00±4.56	91.40±3.28	87.75±7.80	20
	Na^+ (mEq /l)	144.50±5.68	142.80±2.16	142.00±2.19	142.50±2.43	147.33±3.55	20
	K^+ (mEq/l)	5.15±0.87	5.06±1.64	4.15± 1.13	4.90±0.75	6.08±1.00	80
	Cl^- (mEq/l)	103.25±4.57	101.6±3.28	101.16± 1.94	101.83±2.14	105.00±2.56	20
W_6	Ca^{2+} (mg/l)	95.25±1.70	96.33±2.65	97,00±2.44	97.16±0.98	89.16±9.17	20
	Na^+ (mEq /l)	141.75±2.87	142,00± 3,40	143,16± 5,30	148,33± 7,00	145,00± 5,40	20
	K^+ (mEq/l)	5.22±0.97	5.53±1.33	5.00±0.50	4.62±0.46	5.10±0.42	80
	Cl^- (mEq/l)	101.25±1.89	102.16±1.83	101.83±3.37	104.66±5.39	103.66±3.61	20

NB : OVR = % workforce outside values of reference

Table No.3: Decomposition of the variance analyses of the electrolytes with $\alpha = 5\%$

Settings	Sources of variations	ddl	SCE	CM	F_{obs}	F_{th}	F test
Ca^{2+}	Times	5	175.151	35.030	2.051	2.27	Not significant
	Doses injected	1	179.544	179.544	10.511	3.90	Significant
	Inter – action	5	241.635	48.327	2.829	2.27	Significant
	Residual	154	2408.437	17.081			
K^+	Times	5	540.681	108.136	4.904	2.27	Significant
	Doses injected	1	6.950	6.950	0.315	3.90	Not significant
	Inter – action	5	259.821	51.964	2.356	2.27	Significant
	Residual	154	3395.975	22.052			
Na^+	Times	5	17.856	3.571	5.553	2.27	Significant
	Doses injected	1	0.359	0.359	0.558	3.90	Not significant
	Inter – action	5	9.749	1.950	3.032	2.27	Significant
	Residual	154	99.030	0.643			
Cl^-	Times	5	140.259	28.052	1.139	2.27	Not significant
	Doses injected	1	87.024	87.024	3.533	3.90	Not significant
	Inter – action	5	80.204	16.041	0.651	2.27	Not significant
	Residual	154	3793.765	24.635			

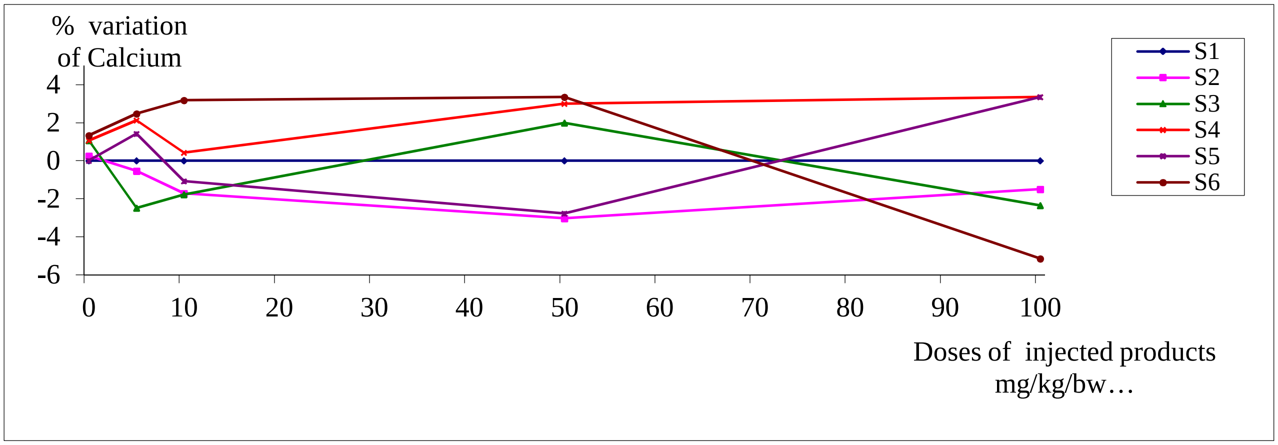

Figure No.1: Variation curve for Ca^{2+}serum according to the doses used in treatments of animals

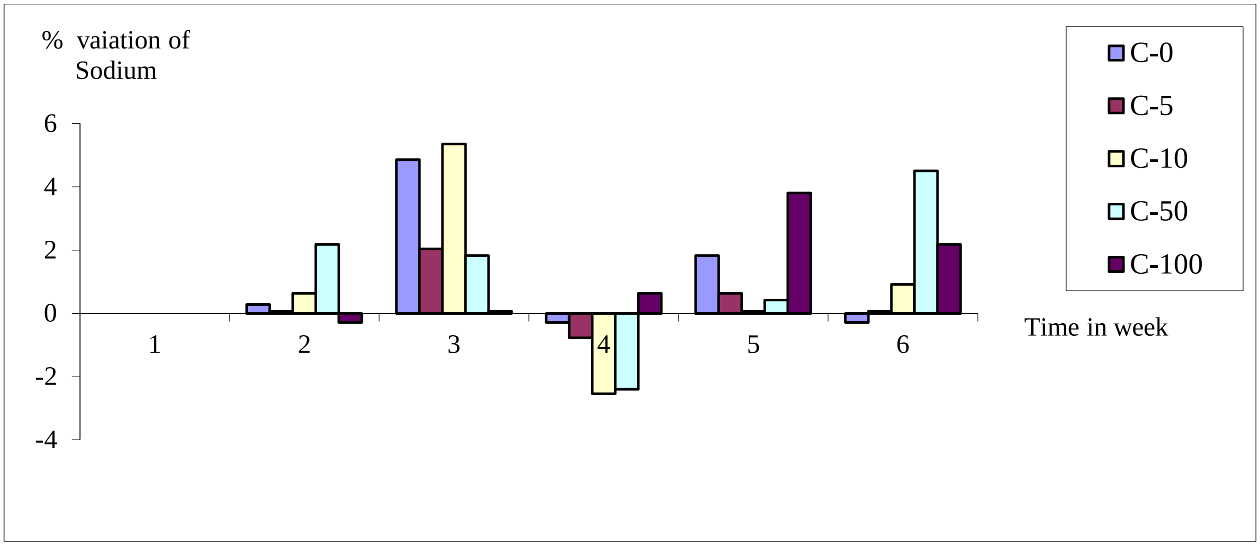

Figure No.2: Histogram of variation for Na^+ serum level according to the duration of the treatment of animals with different doses of the injected products

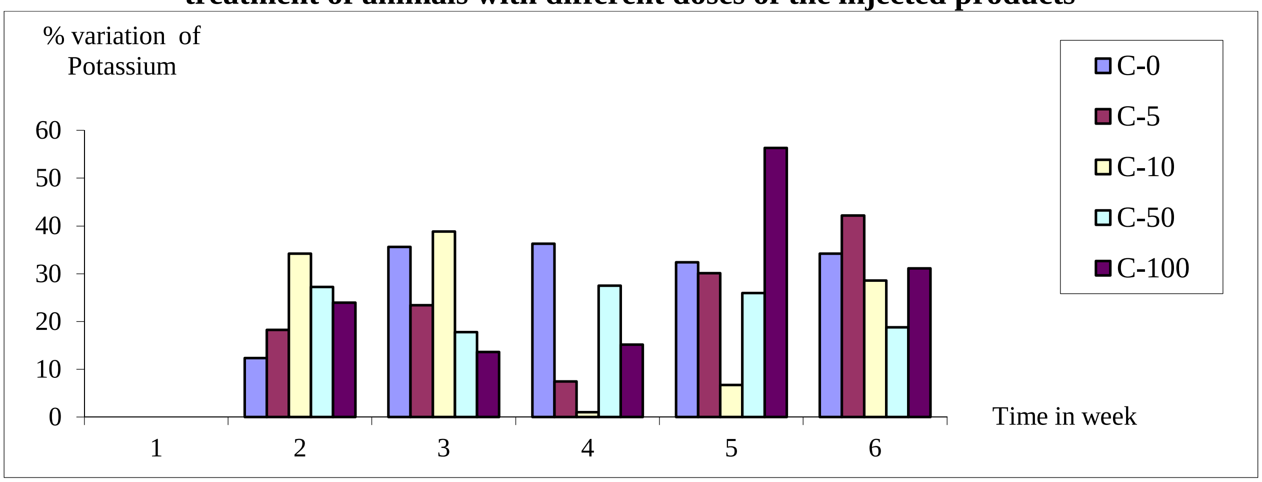

Figure No.3: Histogram of variation for potassium serum level according to the duration of the treatment of animals with different doses of the injected products

CONCLUSION

This work also enabled to monitor electrolyte serum variations used in the rabbits treated with different concentrations of aqueous extracts from *Phyllanthus amarus*. Thus, serum parameters that fluctuated with likely disturbances are for calcium an effect of the doses of the injected products while for sodium and potassium it is rather the product exposure time effect. Such different manifestations of electrolytes in the presence of aqueous extracts from *Phyllanthus amarus* would affect the activity of the cells with repercussions on such vital organs as the heart, kidney and liver. These findings probably confirm that the aqueous extracts from *Phyllan thus amarus* could certainly have actions in the treatment of diabetes and hypertension.

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CONFLICT OF INTEREST
We declare that we have no conflict of interest.

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