

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: www.ijrpns.com



ASSESSMENT OF SERUM ENZYMES OF LIVER IN RABBITS TREATED WITH AN AQUEOUS EXTRACT OF *PHYLLANTHUS AMARUS* (EUPHORBIACEAE)

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ABSTRACT

The *in vivo* assessment of serum markers of the liver in rabbits treated with aqueous extracts of *Phyllanthus amarus* were used to determine the enzymatic activity of α - amylase, Alkaline phosphatase (ALP), Creatine phosphokinase (CPK), Lactate dehydrogenase (LDH), Alanine - aminotransferase (ALAT), Aspartate - aminotransferase (ASAT) and Gamma glutamyl transferase (γ GT). During this experiment, the rabbits were treated intraperitoneally for four weeks (from 0 to 100 mg / kg / body weight) and one week without treatment. Statistically significant changes were observed showing the effect of doses of the extract on α - amylase with reductions in percentage changes from concentrations of 10 mg / kg / body weight whereas percentage increase was observed during longer period of exposure to the product effect from the third week there was percentage increase from 100% to 350 % for ALAT , 50 % to 450 % for ASAT, from 20% to 100 % for ALP and decrease from - 10% to - 60% for LDH. In conclusion the aqueous extracts of *Phyllanthus amarus* would not significantly have toxic effect on the hepatocytes only when they are used for a shorter duration (less than three weeks) with low concentrations (from 5 to 10 mg / kg / body weight).

KEYWORDS

Phyllanthus amarus, Liver and Enzyme activity.

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INTRODUCTION

About 80% of the population in Africa depends on medicinal plants for their treatment. These could be exploited in other to search for new therapeutic molecule. Among these medicinal plants, the aqueous extracts of *Phyllanthus amarus*, a Euphorbiaceae (Shunn and Thom) have been examined, its effect on the serum enzymatic parameters especially in the kidneys has shown that

prolonged use of this drug could possibly cause an organ dysfunction while these aqueous extracts could probably be a cardioprotector^{1,2}.

Apart from the heart and kidneys, an organ like the liver is the subject of this study on the assessment of serum enzymatic parameters in rabbits treated with an aqueous extract of *Phyllanthus amarus*. The liver is mainly constituted of some KUPFFER cells that belong to the endothelial lining of blood vessels and liver cells, the functional entity control all metabolic processes in the body.

By considering foreign substances in the body, which consist mainly of drugs and foods, the predominant pathway is comparable to the substances produced by the body (bile pigments, hormones)³. The main mechanisms involved in the activity of liver cell are biosynthesis, excretion, storage, transformation and degradation.

Abnormalities in the liver due mainly to disturbances in the metabolic pathways as a result of substances having more or less toxic effect⁴. In view of the duration of drug in the body organism, substances are subjected into two types of specific actions which are, formation of soluble compounds ejected from the body through kidneys excretion and developing more or less active metabolite.

The liver damage may be either hepatitis characterized by inflammation of the liver with acute cytolysis. This disturbance was conjugated with chronic cholangitis remarkable by inflammatory interlobular bile ducts in an acute condition. It's also accompanied with a jaundiced after surgery in a chronic condition with the presence of the product causing a progressive destruction of the bile ducts. There is also the case of the liver macromolecular steatosis or micro molecular of phospholipase.

However, hepatic cells which participate in the activities of the body organ by the action of enzymes which produced and reflect specifically the general state of the organism. Those reactions were produced in the presence of aqueous extract of plants such as *Phyllanthus amarus*.

The potential therapeutic activity extracts of *Phyllanthus amarus* was shown in hypertension, diabetes and jaundice. It shows protective action on

hepatocytes⁵⁻⁹. Moreover, the use of the extract of the plant has revealed its relative effectiveness on hepatitis B and HIV^{10,11}.

The liver has enzymatic biomarkers for the detoxification of substances administered into the circulatory system and its damage may cause disturbances on histological, cytological and biochemical metabolism⁴.

In biochemical set up, three essential abnormalities can be observed, the decrease in cellular ATP, modification of the methylation reaction and reduces the disturbance of Glutathione^{12,13}. These abnormalities called hepatitis resulting from large metabolic disorder due to substances having a more or less toxic capacity. They are characterized by acute hepatitis in three specific cases. In the presence of an excess of toxic products, there is cytolysis of hepatocytes characterized by an increase in Alanine - aminotransferase (ALAT) and Aspartate - aminotransferase (ASAT) in the general circulation so when the presence of excess hormones like androgens or estrogens there is cholestasis characterized by the significant increase in parameters such as Gamma - Glutamyl transferase (γ GT), Alkaline phosphatase (ALP), 5'- nucleotides. However there is a mixed form generally characterized by non-steroidal anti- inflammatory. Contrary to chronic hepatitis rarely occurred, and in the presence of products such as α -methyl dopa, papaverine, nonsteroidal anti-inflammatory drugs cause cytolysis characterized by increased ASAT and ALAT in the general circulation.

Classification of enzymes can specifically distinguish plasma and non-plasma¹⁴. Ceruloplasmin, lipoprotein lipase, coagulation enzymes and fibrinolysis are classified as non-plasma while plasma enzymes include excreta synthesized by exocrine glands such as alkaline phosphatase (ALP) and α - amylases and cellular enzymes belonging to all metabolisms are represented by creatine phosphokinase (CPK), lactate dehydrogenase (LDH), alanine - amino transferase ALAT, aspartate - amino transferase ASAT and gamma glutamyl transferase (γ GT) some of these enzymes such as Creatine Phosphokinase

CPK and Lactate Dehydrogenase LDH were subjected to fractionation by electrophoresis to identify their specific impacts on the functioning of the liver^{12, 15, 16}.

These parameters have formed the basis of our study on average values of serum biomarkers in rabbits in tropical areas¹⁷. These values were determined for ALAT (45.52 ± 20.54 IU/L), ASAT (21.24 ± 9.89 IU/L), ALP (432.66 ± 207.8 IU/L), γ GT (24.24 ± 15 21 IU/L), CPK (954 ± 343.4 IU/L), LDH (1135 ± 335.93 IU / L) and finally amylases (114.72 ± 27.99 IU/L).

MATERIALS AND METHODS

The biological materials were the leaves of *Phyllanthus amarus*. We also used 30 rabbits (*Oryctolapus cuniculus*) weighing approximately 2.5 kg of body weight. Two groups of rabbits were formed, 15 males and 15 females. Each group was divided into five groups of three rabbits. The enzymatic parameters assay reagents consisted of a set of kit for CPK, LDH, ALAT, ASAT, ALP, γGT and α -amylases from Biosystem[®]. The assays were performed using an automatic biochemical Hitachi 704.

Preparation Method of medicinal plant

The preparation method of the aqueous extract of *Phyllanthus amarus* for authors¹⁸ consisted of 100 g of dried whole leaves that were soaked in one liter of distilled water, filtered and then lyophilized thus obtaining a final yield of 12 %. Then, 200 mg of dried powder was diluted in one liter of distilled water. Forming a con stock solution of 200 mg / l.

Treatment of animals

The two-month old rabbits were acclimatized for two weeks and fed with granules from Ivograins[®] according to the standard procedures¹⁹. Blood samples were taken for sampling at eight o'clock in the morning, from the ear marginal vein one week before treatment and during the four weeks of intraperitoneal injection with precise dose of 0, 5, 10, 50 and 100 mg / kg of body weight and one week after the last treatment. These doses are administered to rabbits based on the maximum tolerated dose (MTD) in rats which is 125 mg / kg /

bw¹³. The blood samples are centrifuged for five minutes at 3000 RPM, and the sera obtained were frozen at -20 °C preserved for various dosages.

Biochemical Analysis of enzymatic serum parameters

Biological analyzes of different serum parameters of each blood sample were performed on a spectrophotometer. HITACHI 704. Then enzyme kinetics was used in order to determine the activity of ALAT, ASAT, LDH and CPK (spectrometer at 340 nm). On the other hand, the wavelength λ of 405 nm (ALP activity) was determined appearance of Phosphonitrophenol (PNP); while γ GT was determined by the rate of formation of Phosphonitroaniline. At the same wavelength λ (405 nm), the α - amylases was determined by forming the 2-chloro 4 -nitrophenol.

Statistical analysis

For this study the mean and standard deviations for each parameter analyzed was calculated and then using Stat View software, a Fisher's exact test with accuracy P equal to 5 % was used for analysis of variance of two factors taking into account the dose effect of injected product and exposed time effect of injected products. Moreover, the implementation of the various figures will be made possible by using the calculation of percentage change of each parameter at a time and at a specific dose was determined by the percentage change of average value of each parameter based on each concentration of product and the time is represented by Y which is given by the following relationship:

$$\% \text{ of change on average } Y = \frac{X_1 - X_0}{X_0} \times 100$$

X₁ = Parameter value determined before treatment.

X₂ = Parameter value determined based on the concentration of the drug administered at a particular time of treatment.

RESULTS AND DISCUSSION

This work on assessment of serum enzymes of liver in rabbits treated with an aqueous extract of *Phyllanthus amarus* (Euphorbiaceae) has been represented in tables and figures.

The data obtained in Table No.1 showed that the parameters were influenced on one hand by different doses injected to rabbits. Those doses (mg/kg/bw) of the extracts of *Phyllanthus amarus* were: $C_0 = 0$, $C_5 = 5$; $C_{10} = 10$, $C_{50} = 50$; $C_{100} = 100$). On the other hand by period of exposure of animals to the product in our study they were observed for six weeks (S_1 , S_2 , S_3 , S_4 , S_5 , S_6).

Table No.2 represented the analysis of enzymes variances with an accuracy of 5%. The enzymatic activities of parameters significantly change. Based on the time of exposure and different concentration of *Phyllanthus amarus* extracts, those parameters were CPK and γ GT. Those variations were observed during the injection of different doses of *Phyllanthus amarus* in the case of amylases (4.124 Fobs > Fth 2.27). The Figure No.1 represented the variations. The enzymatic activities were significantly variable over time in the case of ALAT (Fobs 7.83 > 2.27 Fth), ASAT (Fobs 3.66 > 2.27 Fth), ALP (Fobs 3.37 > 2.27 Fth), LDH (4.68 Fobs > Fth 2, 27). The Figures No.2-5 represented respectively those variations.

This study has provided information on the enzymatic activity of the studied organ and enables comparisons with earlier references^{12, 14, 16}, also to identify the effect of the aqueous extract of *Phyllanthus amarus* on the hepatocytes. Considering the effect of time of exposure and doses of the aqueous extract, this study showed that the enzymes CPK and γ GT do not significantly change ($P > 5\%$) as shown in Table No.2.

In the case of ASAT and ALAT transaminases which increased significantly on the third week of treatment (from 100% to 350 %) (Figure No.2) and from 50 to 450 % respectively (Figure No.3). Also, compared to the data of previous references^{12, 14, 16}, the *Phyllanthus amarus* treatment caused a slight disturbance in the activity of serum transaminase as shown in (Table No.2). These enzymes take part in all cell metabolisms of the organism. Our results suggested that this increase was due to the hepatocyte membrane break up and facilitated the partial release of its cytoplasmic contents. Their expression in the serum biomarker was characterized

by an important liver dysfunction and is an important factor in the inflammatory process of liver cells. Also, for low concentration (from 5 to 10 mg / kg /bw of aqueous extracts observed for two weeks of treatment, changes in transaminases were not noticed as shown in Figures No. 2 and 3.

For alkaline phosphatase (ALP), their increases varied from 20% to 100 % (Figure No.4) over treatment time and particularly from the third week. This could cause dysfunction with liver damage check (Table No.2). Compared to previous references¹⁶, this data suggested that aqueous extracts of *Phyllanthus amarus* induced the release of the enzyme from the cell to the general circulation. The only explanation to this situation is the phenomenon of transient inflammation due to increases in ALP during treatments. This finding may not be consistent with the work of previous references⁹ about the use of *Phyllanthus amarus* in the treatment of jaundice. A likely effect of this natural product on the body organ could be emphasized considering little changes in ALP when used over a shorter period less than three weeks treatment.

The significant reduction in LDH ranging from - 20% to - 60% from the third week of treatment (Figure No.5) and that compared to previous data³. However, it seemed that these values do not identify an obvious pathology in the liver. This enzyme LDH is present in all cell metabolism¹⁴ and it could have, a reduced activity or inhibition of its synthesis in the cell during treatments with aqueous extracts of *Phyllanthus amarus*. The reduction of the enzyme LDH suggested a possible protective effect of hepatocytes in accordance with the previous work⁹. Those results showed an increase of the survival time of liver cells compared to the references¹¹. In addition, compared to previous work¹⁵ the research of LDH isoenzymes in the presence of *Phyllanthus amarus* expected to locate fractions involved in the hepatic metabolism probably responsible for the biological variations.

Concerning the reduction in the activity of α - amylase from 10 mg / kg body weight (Figure No.1) and confirmed by the statistical analysis in

Table No.2, the comparison with previous data^{12, 17}, suggested an inhibition effect of *Phyllanthus amarus* on either the synthesis or the activity of the enzyme. In addition to the possible protective effect of *Phyllanthus amarus* on liver cells described during

treatment against hepatitis B in previous work¹⁰ is also *in vitro* potential natural products against HIV which require further study¹⁴.

Table No.1: Mean values of the parameters during the experiment

Treatment		Enzymatic Parameters analyse with standard deviation in UI/L						
Times (in weeks)	Doses mg/Kg of bw	ALAT	ASAT	PAL	γ GT	CPK	LDH	Amylase
BFT	C ₀	45.52 ± 20.54	21.24 ± 9.89	456.32 ± 207.08	24.04 ± 15.51	954.20 ± 343.40	1135.16 ± 335.93	114.72 ± 27.99
1 st	C ₀	26.80 ± 12.81	16.75 ± 15.59	512.50 ± 231.40	15.25 ± 10.40	941.00 ± 168.04	1057.00 ± 466.50	98.50 ± 30.14
	C ₅	43.80 ± 19.33	15.80 ± 4.81	581.60 ± 134.00	18.00 ± 2.12	776.50 ± 310.06	986.20 ± 346.11	129.60 ± 28.84
	C ₁₀	49.60 ± 19.51	23.40 ± 19.50	467.20 ± 168.54	17.00 ± 2.16	879.25 ± 158.83	1089.80 ± 495.62	119.00 ± 26.21
	C ₅₀	57.00 ± 39.95	20.40 ± 8.79	457.60 ± 195.84	19.80 ± 11.65	891.40 ± 236.49	1003.80 ± 554.09	100.80 ± 9.60
	C ₁₀₀	42.40 ± 7.92	15.00 ± 4.69	601.20 ± 211.60	13.20 ± 1.64	845.80 ± 374.14	1225.20 ± 534.33	121.80 ± 28.27
2 nd	C ₀	48.66 ± 14.22	22.66 ± 3.51	489.66 ± 106.51	14.66 ± 1.15	989.66 ± 363.79	1223.33 ± 372.25	93.33 ± 5.50
	C ₅	48.60 ± 21.37	32.40 ± 15.18	593.00 ± 140.54	16.20 ± 6.26	778.00 ± 430.55	1331.00 ± 926.84	109.66 ± 33.69
	C ₁₀	95.00 ± 48.53	58.00 ± 38.09	536.83 ± 212.81	23.33 ± 21.29	828.50 ± 337.38	1299.16 ± 313.10	106.33 ± 30.97
	C ₅₀	37.00 ± 18.33	25.40 ± 26.74	469.00 ± 233.38	14.80 ± 1.09	558.80 ± 368.62	811.40 ± 368.61	89.00 ± 11.71
	C ₁₀₀	108.40 ± 115.63	80.20 ± 62.88	634.40 ± 252.65	15.40 ± 5.08	640.20 ± 454.57	1137.00 ± 802.31	90.40 ± 19.78

3 rd	C ₀	107.66 ± 42.16	59.33 ± 32.00	822.00 ± 48.44	44.66 ± 60.34	603.00 ± 325.75	520.66 ± 279.72	110.66 ± 16.44
	C ₅	101.50 ± 70.38	56.33 ± 47.5	707.00 ± 155.39	11.00 ± 5.65	668.50 ± 486.77	817.66 ± 484.43	143.66 ± 62.34
	C ₁₀	123.83 ± 88.32	79.33 ± 61.56	731.66 ± 140.63	50.50 ± 63.41	559.33 ± 469.50	602.50 ± 236.52	119.00 ± 30.65
	C ₅₀	75.66 ± 39.88	66.16 ± 32.09	722.33 ± 276.72	45.40 ± 59.13	1049.00 ± 269.33	429.00 ± 165.46	104.50 ± 15.46
	C ₁₀₀	125.33 ± 89.94	114.83 ± 62.64	917.66 ± 460.34	16.00 ± 8.57	629.00 ± 485.34	576.80 ± 224.83	107.16 ± 16.97
4 th	C ₀	130.33 ± 69.18	63.66 ± 42.45	791.66 ± 219.40	8.00 ± 2.50	557.33 ± 398.10	595.33 ± 443.02	153.00 ± 73.74
	C ₅	156.50 ± 190.28	54.00 ± 69.87	559.50 ± 119.62	33.00 ± 20.22	604.00 ± 327.37	841.16 ± 372.41	158.00 ± 97.28
	C ₁₀	182.66 ± 154.23	104.16 ± 94.04	624.50 ± 69.19	14.33 ± 7.57	904.66 ± 295.09	1013.67 ± 211.68	118.83 ± 30.70
	C ₅₀	52.00 ± 13.94	20.60 ± 6.10	469.20 ± 47.55	12.00 ± 1.41	727.60 ± 366.18	636.20 ± 350.65	122.60 ± 31.13
	C ₁₀₀	41.80 ± 12.96	19.80 ± 3.19	623.60 ± 196.06	9.33 ± 6.63	794.00 ± 334.56	901.00 ± 318.38	901.00 ± 318.38
AFT	C ₀	59.50 ± 11.03	33.75 ± 13.30	831.50 ± 168.19	21.33 ± 11.59	742.25 ± 552.26	815.00 ± 309.93	133.25 ± 21.40
	C ₅	43.00 ± 26.29	26.16 ± 12.32	641.00 ± 148.02	16.33 ± 4.88	750.00 ± 152.26	804.16 ± 235.13	148.50 ± 77.78
	C ₁₀	41.00 ± 6.56	22.20 ± 4.15	691.00 ± 181.34	22.83 ± 12.99	576.80 ± 200.33	676.60 ± 177.44	117.50 ± 18.40
	C ₅₀	69.16 ± 41.96	37.50 ± 24.78	571.00 ± 158.97	15.16 ± 6.40	632.17 ± 486.18	649.66 ± 272.22	110.00 ± 43.03
	C ₁₀₀	80.33 ± 53.07	50.80 ± 32.76	711.83 ± 168.50	31.66 ± 29.29	860.33 ± 508.28	815.00 ± 361.12	102.80 ± 27.94
BFT: before treatment - AFT: stopping treatment								

Table No.2: Summary of statistical analyzes of parameters with precision p < 5%

Parameters	Source of Variation	Ddl	F _{obs}	F _{th}	Test
ALAT	Time	5	7.827	2.27	Significant
	Injected doses	1	0.943	3.90	Not significant
	Inter – action	5	3.647	2.27	Significant
	Résidual	145			
ASAT	Time	5	3.658	2.27	Significant
	Injected Doses	1	0.001	3.90	Not significant
	Inter – action	5	2.791	2.27	Significant
	Residual	144			
PAL	Time	5	3.371	2.27	Significant
	Injected Doses	1	0.715	3.90	Not significant
	Inter – action	5	0.517	2.27	Not significant
	Residual	145			
γ GT	Time	5	0.981	2.29	Not significant
	Injected Doses	1	0.077	3.92	Not significant
	Inter – action	5	0.677	2.29	Not significant
	Residual	123			
CPK	Time	5	2.066	2.27	Not significant
	Injected doses	1	0.367	3.90	Not significant
	Inter – action	5	0.786	2.27	Not significant
	Residual	139			
LDH	Time	5	4.682	2.27	Significant
	Injected Doses	1	0.052	3.90	Not significant
	Inter – action	5	0.779	2.27	Not significant
	Residual	143			
Amylase	Time	5	1.874	2.27	Not significant
	Injected Doses	1	4.124	3.90	Significant
	Inter – action	5	0.483	2.27	Not significant
	Residual	141			

ddf: degree of freedom

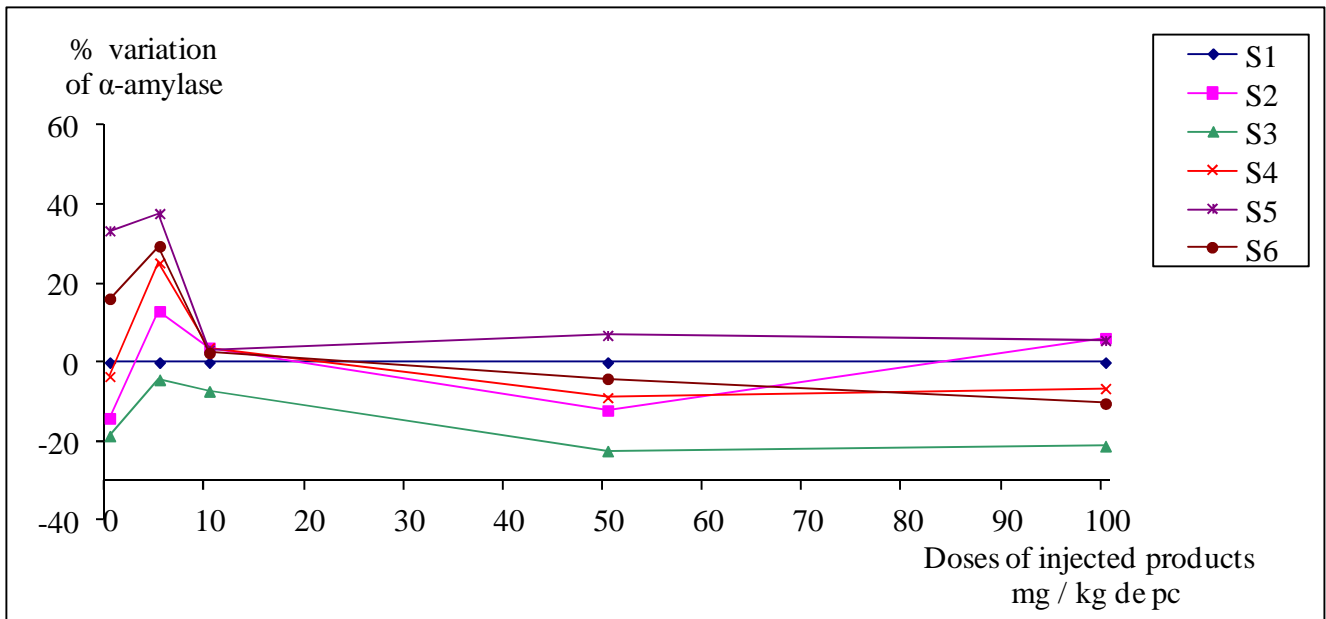


Figure No.1: Percentage changes in the α -amylase according to the doses of injected products each week

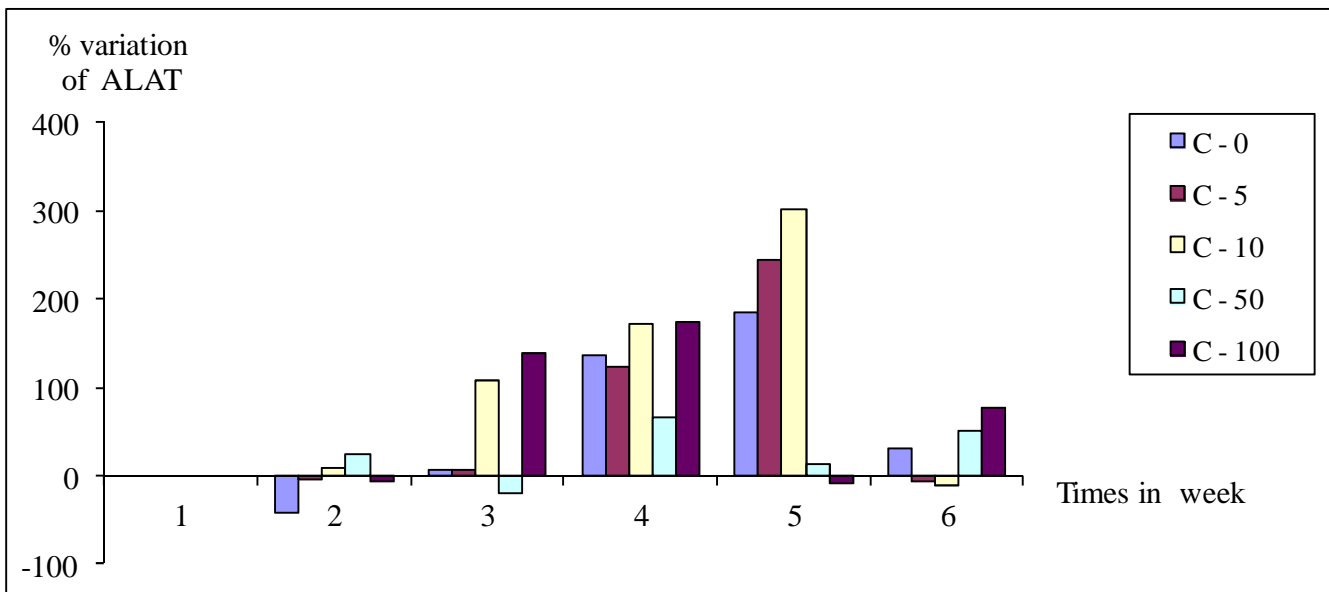


Figure No.2: Percentage changes in ALAT according to time during the week for each dose injected

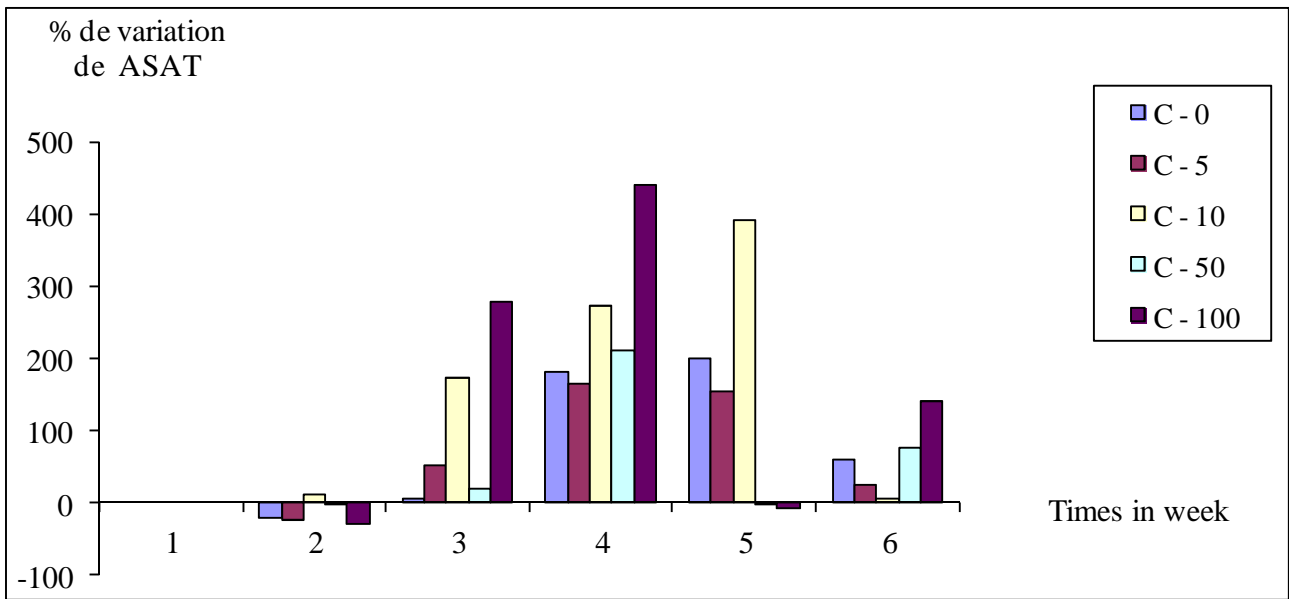


Figure No.3: Percentage change in ASAT according to time during the week for each dose injected

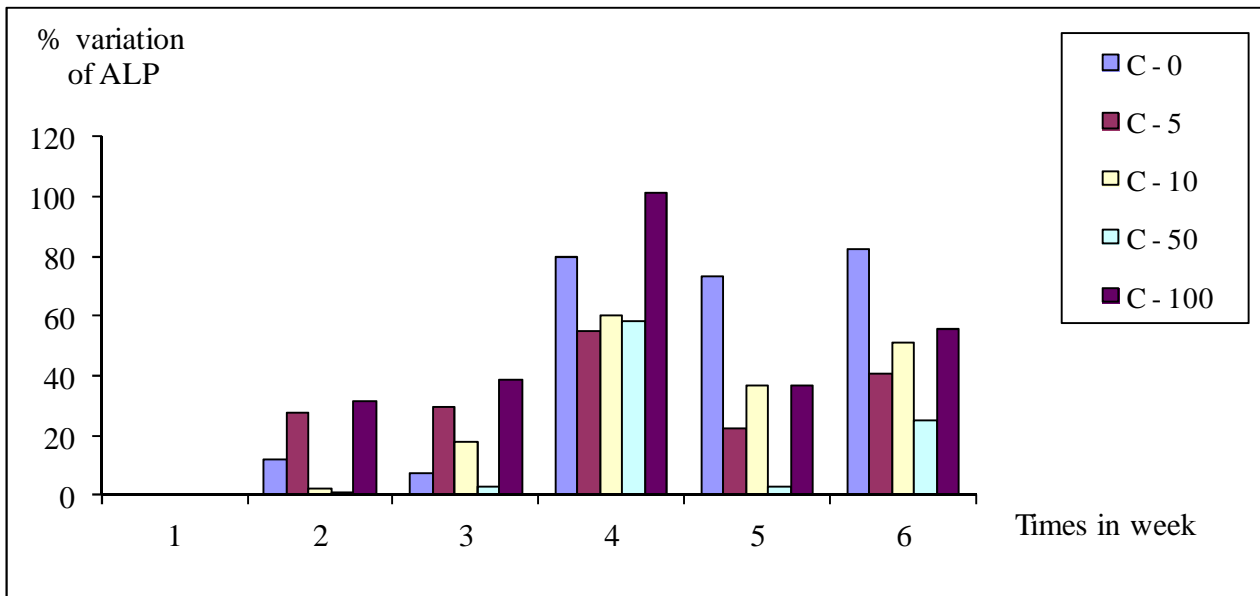


Figure No.4: Percentage changes in ALP base on time in Week for each dose of injected

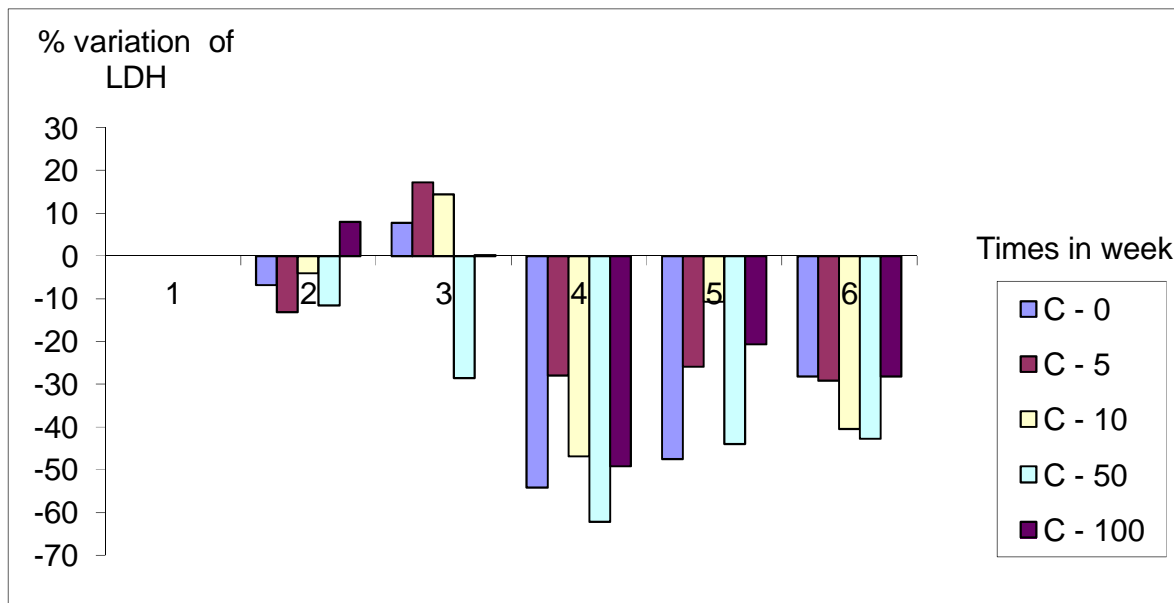


Figure No.5: Percentage changes in LDH base on time in Week for each dose injected

CONCLUSION

This experiment allowed us to follow the temporal variations in the parameters studied in rabbits treated with different concentrations of aqueous extracts of *Phyllanthus amarus*. Thus, the enzymatic parameters showed statistically significant reduction changes due to the effect of doses of the product on the α - amylase on one hand and on the other hand the effect of time of exposure to the products from the third week, an increase of ALAT, ASAT, ALP and reduced LDH. These variations indicate that with the aqueous extracts of *Phyllanthus amarus* used in concentration below 10 mg / kg BW on shorter period of less than three weeks, it would be of beneficial effects on organs such as the liver. This confirmed that aqueous plant extract as natural substance and having medicinal properties particularly actions on the survival of the liver cells. Among these properties, the study of the uses of the product in the treatment of hepatitis B and HIV would be considered taking into account the extent of the disease in the world.

ACKNOWLEDGEMENT

We would like to thanks the Laboratory of Pharmacology and Biochemistry, UFR Biosciences, University Felix Houphouet-Boigny, Abidjan, Cote d'Ivoire for the continuous support and encouragement throughout this work.

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