Scientific Research Publishing

American Journal of Molecular Biology, 2025, 15(1), 25-38
https://www.scirp.org/journal/ajmb
ISSN Online: 2161-6663
ISSN Print: 2161-6620

Cytotoxicity and Hepatic Effects *in Vivo* of the Phytomedicinal Antipalu Used by the Population of Abidjan

Kouakou Serge Kouassi[1*], Aka Edwige Ayebe[2], Irié Lou Bohila Emilie Kamo[3], Djenabou Kamagate[3], Ouagnonan Ismael Soro[3], Gnogbo Alexis Bahi[1], Allico Joseph Djaman[1,3]

[1]Biology and Health Laboratory, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire
[2]Department of Clinical and Fundamental Biochemistry, The Institut Pasteur de Côte d'Ivoire, Abidjan, Côte d'Ivoire
[3]Department of Microbiology, The Institut Pasteur de Côte d'Ivoire, Abidjan, Côte d'Ivoire
Email: *ksergecci@yahoo.fr, *kouassi.serge@ufhb.edu.ci

How to cite this paper: Kouassi, K.S., Ayebe, A.E., Kamo, I.L.B.E., Kamagate, D., Soro, O.I., Bahi, G.A. and Djaman, A.J. (2025) Cytotoxicity and Hepatic Effects in Vivo of the Phytomedicinal Antipalu Used by the Population of Abidjan. *American Journal of Molecular Biology*, **15**, 25-38.
https://doi.org/10.4236/ajmb.2025.151003

Received: October 12, 2024
Accepted: December 3, 2024
Published: December 6, 2024

Abstract

Antipalu is a phytomedicinal medicinal beverage that is popular in the District of Abidjan, particularly for the treatment of malaria. However, Antipalu could present potential health effects on patients, and few toxicological studies have been conducted before its use. In order to determine the cytotoxicity of Antipalu, two complementary tests, LDH activity and the MTT cell proliferation assay, were used using Vero cells. Vero cells were exposed to increasing concentrations of Antipalu and incubated for 24, 48 and 72 hours. In addition, forty (40) rats distributed randomly into 4 groups, including 10 animals per group (5 males and 5 females) were used for the potential hepatoxic effects. Animals in group 1 received distilled water and were used as a control group. On the other hand, Lot I, II and III received by gavage a volume of the Antipalu extract corresponding to 1 ml/100 g of body weight at 200 mg/kg, 400 mg/kg, 800 mg/kg, respectively. The extract was administered daily at the same time for 28 days and serum was collected once a week to evaluate hepatic biochemical markers. After 28 days of study, all rats were euthanized by an overdose of ether and the liver of the rats was removed for gross morphological and histopathological analysis. The results of the cell supernatant assay showed an increasing extracellular LDH enzyme activity with lethal concentrations at 10% and 50% (LC_{10} = 111 µg/mL and LC_{50} = 555 µg/mL, respectively). In addition, the MTT assay showed a decrease in mitochondrial activity and thus cell proliferation after 24, 48 and 72 H of incubation. Our study showed that Antipalu caused alterations in the plasma membranes of the cells, resulting in the release of lactase dehydrogenase (LDH) into the external environment and a decrease in the mitochondrial activity of the Vero cells. The

biochemical parameters ALT, ASAT, ALPs, and GGT showed no significant change ($P > 0.05$) in the group of treated rats compared to the controls. However, these variations were moderate and transient, with values remaining almost within their standard limits. Microscopic observations of liver tissue sections from rats treated with the Antipalu showed no lesions, edema and necrosis. These results suggest that the Antipalu did not interfere with the functioning or alter the integrity of the liver.

Keywords

Phytomedicinal, Antipalu, Cytotoxicity, Vero Cells, Hepatic Biomarkers

1. Introduction

The use of medicinal plants for therapeutic purposes is gaining increasing interest among the world population, and they have become highly coveted products in both traditional and modern medicine [1]. They are widely used in traditional African pharmacopoeia for the prevention or elimination of numerous infections [2] [3]. In fact, around 80% of rural populations in developing countries, especially in sub-Saharan Africa, use medicinal plants as their main means of treating various pathologies [4]. The use of phytomedicines is reflected in their very affordable prices compared with modern medicines, and the proven efficacy of certain medicinal plants in the treatment of several benign or chronic diseases [5]. In Côte d'Ivoire, as in many sub-Saharan African countries, traditional medicine is an age-old practice, and hundreds of medicinal plants are used by local populations for their anti-malarial and anti-inflammatory properties [6].

However, the traditional use of all these plants for therapeutic purposes in no way guarantees their safety. Phytomedicines contain several interacting bioactive compounds, some of which may have beneficial or harmful effects on health [7].

In fact, work on plants of the genus *Aristolochia* used as phytomedicines has revealed the presence of aristolochic acid, a powerful carcinogen and nephrotoxicant [7].

Moreover, certain factors, such as high doses and the combination of several medicinal plants in traditional preparations, make it difficult to predict the toxic effect of the mixture [8] [9]. As with many other phytomedicines, Antipalu is a medicinal drink sold in the Abidjan district and used by the population for the oral treatment of many infections such as malaria [8] [10]. However, to date, no studies have been carried out on the potential health effects of Antipalu. Therefore, it seems appropriate to carry out toxicological research aimed at clarifying the biosafety and safety of Antipalu phytomedecinal, in order to allow its more rational and reassuring use. The present study aims to explore the cytotoxicity effects of Antipalu phymedicinal on certain biochemical markers of rat's liver tissues.

2. Materials and Methods

2.1. Plant Material

The plant material was lyophilized using the Antipalu phytomedicine obtained by freeze-drying from a commercial solution sold in the District of Abidjan. This phytomedicinal had been collected during the ethnomedicinal survey carried out between July and September 2020 in three municipalities of the Autonomous District of Abidjan.

2.2. Experimental Animals

The animal selection was in accordance with the Organization of Economic Cooperation and Development (OECD) guidelines no. 423 [10]. Healthy, young, and nulliparous, non-pregnant Wistar rats that weigh 100 - 120 mg, were 8 - 10 weeks old, and were obtained from the animal house of pharmaceutical science, Abidjan (Côte d'Ivoire) was selected. The animals are picked randomly, and marked to permit individual identification. Animals were kept in plastic cages with wood shavings that were changed every other day for 5 days before dosing. This allows animals to acclimatise to laboratory conditions (ambient temperature 25˚C ± 3˚C; humidity ranged from 35% to 60%; light and dark period, 12/12 hours, bedding cleaned and sterilized). All animals had a regular supply of drinking water and food [11].

2.3. Cells and Culture Conditions

Vero cells derived from African green monkey *Certopithecus aethiops* cell line of renal origin: cell Vero (ATCC number, CCL-81) and have been found in various research as toxicology [12] [13]. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and were kept in a 5% CO_2 incubator at 37˚C. The culture medium was replaced two times per week and the cells were sub-cultured using trypsin-ethylenediamine tetra acetic acid (EDTA) once a week.

2.4. Sampling of Culture Supernatants and Cells for the Study of the Cytotoxicity

Briefly, Vero cells were seeded in 96-well culture microplates (Costar; Fisher Scientific Labosi SAS) at a density of 1×10^4 cells per 200 µl culture media and incubated at 37˚C in a humidified atmosphere containing 5% CO_2. Non-exposed cells were used as negative controls and Triton X-100 (2%, v/v)-exposed cells as positive controls. Accordingly, 16 replicates were chosen at random as control cells (*i.e.*, incubated only with cultured media), eight replicates per Antipalu concentration as exposed cells (*i.e.*, incubated with culture media containing 3.9, 7.81, 15.62, 31.25, 62.5, 125, 250, 500 or 1000 µg Antipalu/ml)) and eight replicates as positive controls. After 24, 48 or 72 h of incubation, Antipalu cytotoxicity in Vero cells was studied using two methods: the determination of the activity of extracellular LDH in cell-free culture supernatants (Cytotoxicity Detection Kit LDH,

Roche Diagnostics) and MTT tetrazolium salt-based colorimeric assay with pertinence for the estimation of metabolic activity of viable cells. Both the effect concentrations (EC) at 10% (EC10) and at 50% (EC50) values were calculated based on the results arising from the most MTT cytotoxicity test

2.5. Treatment with Plant Material

A repeated oral dose of toxicity study was carried out according to the OECD Guideline 407 [8]. The rats were divided into four groups of 10 animals each (5 males and 5 females). Group 1 received 1 ml/100g body weight of distilled water and served as the control group. Groups I, II, and III received extract doses of 200, 400, and 800 mg/kg body weight, respectively. Mortality, body weight, food and water consumption, as well as observation for general toxicity signs of the animals were evaluated daily for 28 days.

2.6. Blood Sample and Organ Collection

At the end of each week, the animals were anesthetized with diethyl ether. The blood was drawn through cardiac puncture and collected in sterile tubes without anticoagulant. Plasma was obtained in one set by centrifuging the blood at 3000 revolutions/min for 10 min and stored at −20˚C in Eppendorf bottles until it required enzymatic activities and concentration of biochemical metabolites assays. The liver was collected and fixed with 10% buffered formalin for further analysis [14] [15].

2.7. Determination of Hepatic Markers of the Rat

Hepatic enzyme activities were determined using a Cobas C311® HITACHI biochemistry system (Roche Diagnostics, France). Tests were performed using commercial kits (Roche Diagnostics, France) based on the manufacturer's instructions, summarized in Table 1.

Table 1. Operating parameters for the quantitative determination of serum cardiac markers.

Hepatic biochemical markers	Methods (Spectrophotometry)	Wavelength (nm)
Alanine aminotransferase (ALT)	Absorption kinetics (Disappearance of NADH)	340
Aspartate aminotransferase (AST)	Absorption kinetics (Disappearance of NADH)	340
Gamma-glutamyltransferase (GGT)	Rate of 2-nitro-5-aminobenzoic acid formation	405
Alkaline phosphatase (ALP)	Absorption kinetics (Rate of p-nitrophenol)	405

2.8. Preparation of Tissue Sections and Histopathology

The hepatic tissues were cut into transverse blocks. An automatic processor (RH-12EP Sakura, Fine Technical Co. Ltd., Tokyo, Japan) was used to further process the blocks. About 12 hours were required for dehydration (96% alcohol for one hour × four changes, and 100% alcohol for one hour × one change).

Clearing was done in three changes of toluene for one hour each. Tissues were impregnated in two changes of paraffin wax with a melting point of 50˚C for a period of 2 hours. Embedding of tissue was done in paraffin using L-shaped metallic moulds. These blocks were put in the refrigerator for a period of 4 - 6 hours. Each block was cut on a rotary microtome (MicromGmbh, Waldorf, Germany). About 5-micrometer-thick tissue section was obtained and placed in the water bath with a temperature of 50˚C below the melting point of paraffin wax. The cut ribbons of tissues were placed on the albumenized glass slide. The sample slides were subsequently stained with haematoxylin-eosin (HE) and examined under a light microscope; photomicrographs of the samples were recorded [11] [16].

2.9. Statistical Analysis

The results are presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) with repeated measures was employed to compare the results according to the administered doses and times of treatment. ANOVA was considered significant when the level of probability (P) was < 0.05; if P < 0.01, this difference is considered as incredibly significant; if highly significant, P < 0.001.

3. Results

3.1. Antipalu Cytotoxicity in Vero Cells

3.1.1. Extracellular LDH Activity

The results of the determinations performed on cell culture supernatants (**Figure 1**) show an increase in extracellular LDH activity on cells exposed to different concentrations and at different exposure times 24, 48 and 72 H. This increase was non-significant after 24 H incubation, except for Antipalu concentrations of 62.5 µg/ml and 1000 µg/ml, where a tendency to increase was observed (P < 0.05). On the other hand, highly significant increases (P < 0.001) in extracellular LDH activity on cells exposed to different concentrations were observed after 72 h of exposure.

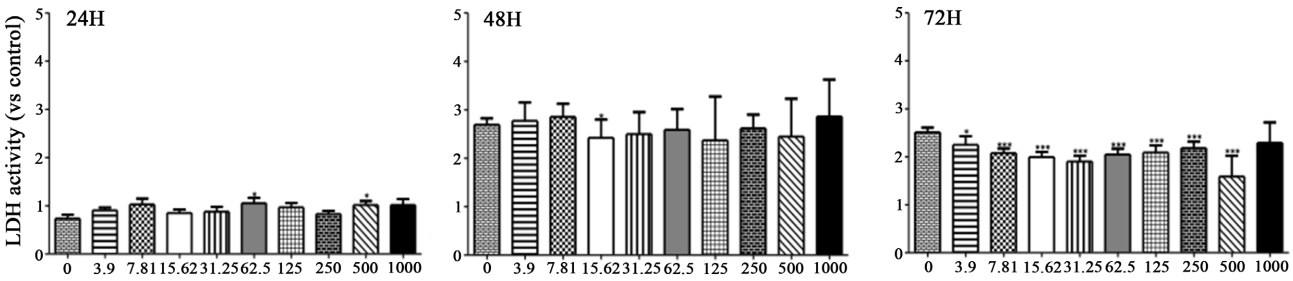

Figure 1. Extracellular lactate dehydrogenase (LDH) activity in cell-free culture supernatants of Antipalu cells. Extracellular LDH activity was measured in supernatants of Vero cells exposed for 24, 48 or 72 h in the continuous presence of increasing concentrations of Antipalu; 3.9, 7.81, 15.62, 31.25, 62.5, 125, 250, 500 or 1000 µg/ml) without renewing the culture media. Non-exposed cells were used as negative controls. Values are depicted as mean values and standard deviation of 16 replicates for negative controls and eight replicates for every Antipalu concentration (percentage vs controls) (Mann-Whitney U-test; vs controls; *P < 0.05; **P < 0.01; ***P < 0.001).

3.1.2. MTT Cell Proliferation Test and Determination of 10 and 50% Inhibitory Concentrations (IC_{10} and IC_{50})

Figure 2 shows MTT results. The results show a tendency towards a decrease in cell proliferation or viability of Vero cells exposed to Antipalu at incubation times of 24, 48 and 72 hours. This decrease is significant and is observed after 24 H for concentrations between 3.9 mg/mL and 125 µg/ml. Similarly, for concentrations between 250 mg/mL and 1000 µg/ml and from 7.81 mg/mL to 1000 µg/ml respectively after 48 h and 72 h exposure to Antipalu, cell viability decreased significantly ($P < 0.01$). However, for concentrations between 3.9 mg/mL and 125 µg/ml after 48 h exposure, the viability of Véro cells decreased, but not significantly ($P > 0.05$).

The proliferation of exposed Vero cells compared with controls measured by MTT assay enabled us to determine the inhibition concentrations 10% IC_{10} and 50% IC_{50} = 555 µg/mL of Antipalu (Figure 3). These 10% and 50% inhibition concentrations are respectively (IC_{10} = 111 µg/mL,) and 50% (IC_{50} = 555 µg/mL).

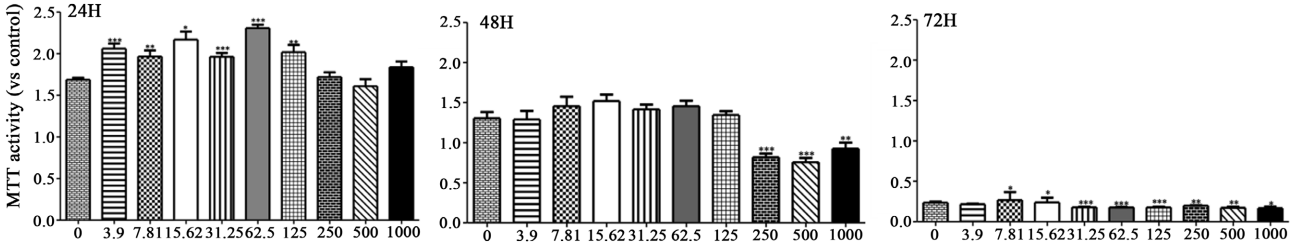

Figure 2. MTT activity in cell-free culture of Antipalu cells. MTT activity was measured in supernatants of Vero cells exposed for 24, 48 or 72 h in the continuous presence of increasing concentrations of Antipalu; 3.9, 7.81, 15.62, 31.25, 62.5, 125, 250, 500 or 1000 µg/ml) without renewing the culture media. Non-exposed cells were used as negative controls. Values are depicted as mean values and standard deviation of 16 replicates for negative controls and eight replicates for every Antipalu concentration (percentage vs controls) (Mann-Whitney U-test; vs controls; *P < 0.05; **P < 0.01; ***P < 0.001).

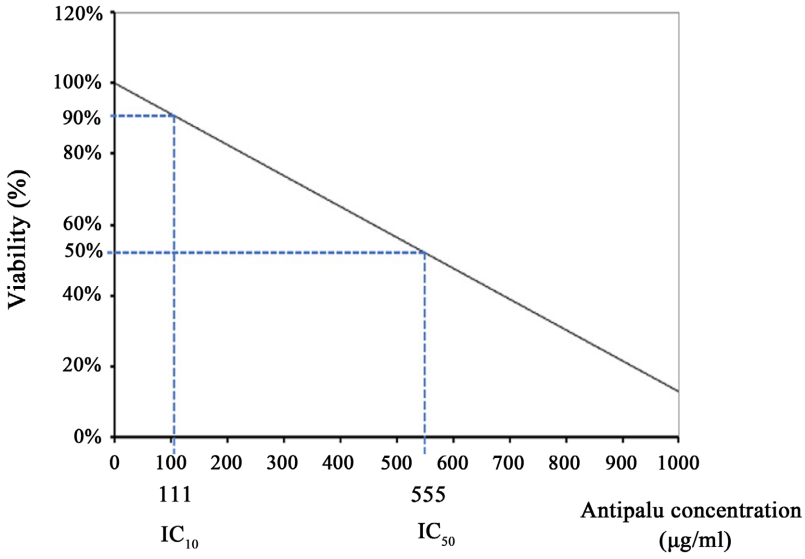

Figure 3. Determination of inhibition concentrations (IC) of Antipalu samples in Vero cells. The more sensitive cytotoxicity test was the quantification of MTT activity in Antipalu-exposed cells. Results arising from MTT activity determination allowed the determination of the IC at 10% (IC_{10}) and at 50% (IC_{50}) for each Antipalu sample.

3.2. Biochemistry Results

3.2.1. Alanine Aminotransferase (ALAT)

Figure 4 shows the ALT activity of rats exposed to Antipalu extract (Table 2). Mean ALAT activity values at the start of the experiment (J0) were 228.65 ± 21.85 IU/L in Lot T and 193 ± 38.48 IU/L to 326.20 ± 63.40 IU/L for Lots I to III respectively. After 14 days of treatment with Antipalu extract, a decrease in ALAT activity was observed in Lot I. Similarly, after 28 days of treatment, ALAT activity decreased in treated rats. However, this was not significant significantly ($P > 0.05$). Mean values for ALAT activity ranged from −15.59% to 42.66% (D0); −25.77% to 0.8% (D14); 35.51 to 84.45% (D28) compared with the control Lot.

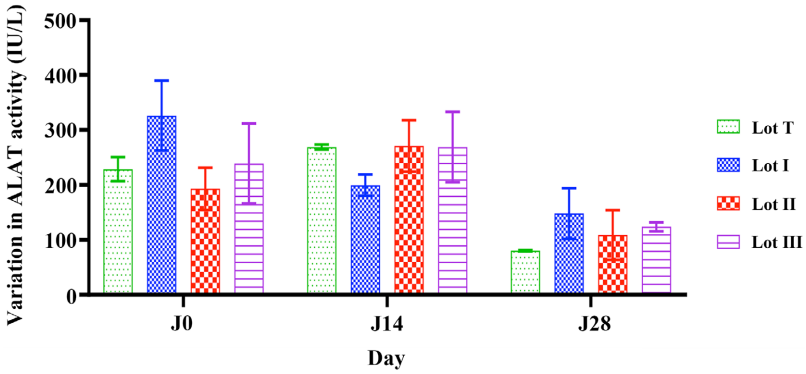

Figure 4. Effects of Antipalu on ALT activities as a function of time. Each bar represents the mean ± standard deviation, n = 10 with Lot T = control; Lot I = 200 mg/kg; Lot II = 400 mg/kg; Lot III = 800 mg/kg of body weight of the animal; the asterisk indicates the significant differences of each group of animals treated according to the time of each week (*$P < 0.05$).

Table 2. Summary of biochemistry values.

		Lot T	Lot I	Lot II	Lot III
ASAT	Initial	289.3 ± 9.4	383.88 ± 96.57	287.48 ± 15.36	280.26 ± 35.29
	P-value		0.370	>0.999	0.997
	J14	192.35 ± 12.15	179.28 ± 12.08	245.40 ± 22.39	199.50 ± 12.22
	P-value		0.994	0.790	0.999
	J28	222.2 ± 19.5	269.23 ± 30.96	309.65 ± 90.15	229.83 ± 11.21
	P-value		0.826	0.556	0.999
ALAT	Initial	228.65 ± 21.85	326.20 ± 63.40	193 ± 38.48	239.04 ± 72.75
	P-value		0.422	0.922	0.997
	J14	268.8 ± 4.6	199.53 ± 19.42	270.97 ± 46.62	268.97 ± 64.31
	P-value		0.674	>0.999	>0.999
	J28	80.25 ± 1.35	148.03 ± 45.94	108.75 ± 45.15	123.87 ± 8.13
	P-value		0.697	0.975	0.900

Continued

GGT	Initial	1.5 ± 1.5	2.25 ± 1.31	5.25 ± 1.49	4 ± 0.71
	P-value		0.965	0.212	0.496
	J14	2.5 ± 1.5	2 ± 1.08	4.67 ± 1.86	3.33 ± 0.67
	P-value		0.990	0.646	0.963
	J28	3 ± 2	2.75 ± 1.55	2 ± 1	5.33 ± 2.03
	P-value		>0.998	0.955	0.607
PAL	Initial	521.5 ± 125.5	483.5 ± 35.42	547.25 ± 104.55	705 ± 84.62
	P-value		0.961	0.987	0.213
	J14	831.5 ± 3.5	656.75 ± 28.81	680.67 ± 45.67	895.67 ± 82.89
	P-value		0.252	0.395	0.879
	J28	594 ± 74	424.25 ± 39.18	418.5 ± 106.5	530.33 ± 44.80
	P-value		0.278	0.358	0.887

3.2.2. Aspartate Aminotransferase (ASAT)

Figure 5 shows the influence of Antipalu phytomedicinal extract on ASAT activity (Table 2). The results of ANTIPALU's influence on ASAT activity are shown in Figure 5. At J0, mean ASAT activity values were 289.30 ± 9.40 IU/L in Lot T, while they ranged from 280.26 ± 35.29 IU/L to 383.88 ± 96.57 IU/L in treated lot animals. On day 14, ASAT activity increased in Lot II and Lot III treated animals and remained at 229.83 ± 11.21 IU/L and 309.65 ± 90.15 IU/L until the end of treatment. However, this increase was not significant ($P > 0.05$. Rates of change in ASAT activity were obtained from mean values and ranged from −3.12% to 32.69% (J0); −6.79% to 27.58% (J14); 3.43% to 39.35% (J28) compared with the control lot.

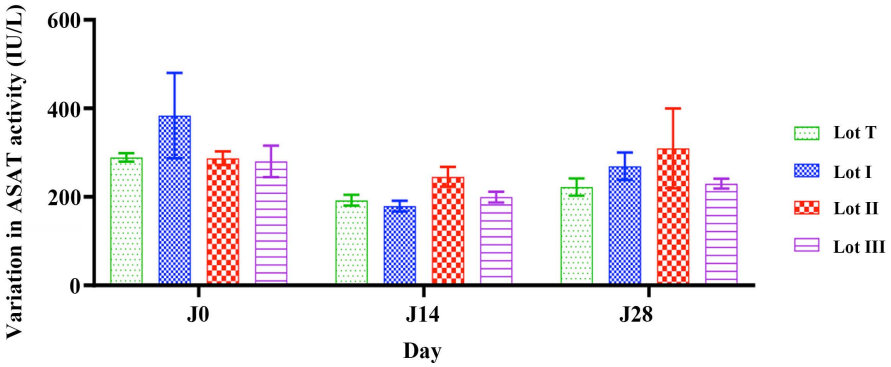

Figure 5. Effects of Antipalu on ASAT activity as a function of time. Each bar represents the mean ± standard, n = 10 with Lot t = control; Lot I = 150 mg/kg; Lot II = 300 mg/kg; Lot III = 600 mg/kg of body weight of the animal; S1; S2; S3; S4: weeks of study. The differences observed between batches and over time are not significant: $P > 0.05$.

3.2.3. Alkaline Phosphatases (ALPs)

Figure 6 shows the influence of Antipalu extract on ALPs activity (Table 2). ALPs activity did not vary significantly ($P > 0.05$) in the treated animals compared to the control animals. However, depending on the evolution over time, we observe a slight increase ($P > 0.05$) in the ALPs activity of Lot III 895.67 ± 82.67 (J14). Likewise, ALPs activity decreased in rats from batch III from 895.67 ± 82.67 (J14) to 530.33 ± 44.80 UI/L (J28), which corresponded to variations of −19.96%; −15.74%; −12.86% and −15.42% decrease.

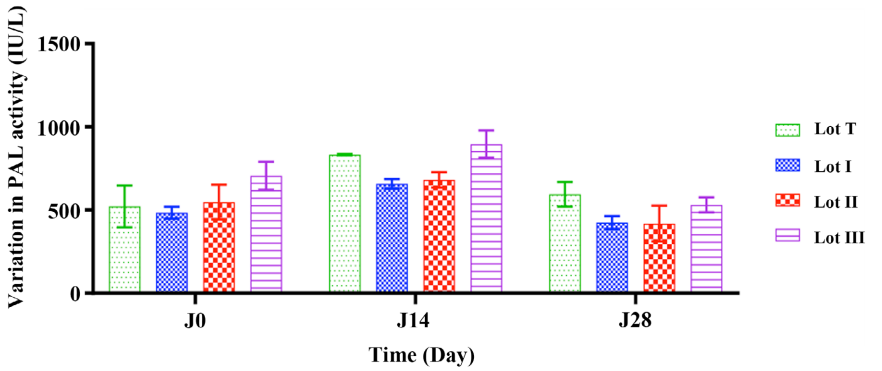

Figure 6. Effects of Antipalu on ALPs activity as a function of time. Each bar represents the mean ± standard, n = 10 with Lot T = control; Lot I = 150 mg/kg; Lot II = 300 mg/kg; Lot III = 600 mg/kg of body weight of the animal; S1; S2; S3; S4: weeks of study; the asterisk indicates the significant differences of each group of animals treated according to the time of each week (*$P < 0.05$).

3.2.4. Gamma-Glutamyl Transférase (GGT)

Figure 7 shows the influence of Antipalu phytomedinal extract on GGT activity (Table 2). Mean GGT activity values on treatment day 0 were 1.50 ± 1.5 IU/L in lot T, while they ranged from 2.25 ± 1.31 IU/L to 5.25 ± 1.49 IU/L in the treated lots. At J14, these values were 2.5 ± 1.5 IU/L in batch T, while they ranged from 2 ± 1.08 IU/L to 4.67 ± 1.86 IU/L in treated lots. At J28, GGT activity values were 2 ± 1 IU/L

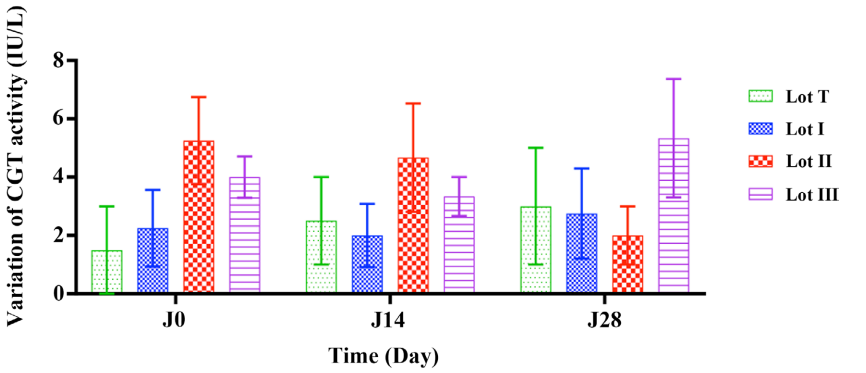

Figure 7. Effect of Antipalu on GGT activity according to the time. Each bar represents the mean ± standard, n = 10 with Lot T = control; Lot I = 200 mg/kg; Lot II = 400 mg/kg; Lot III = 800 mg/kg of body weight of animal Jo; J14 and J28: days of study; the asterisk indicates the significant differences of each group of animals treated according to the time of each week (*$P < 0.05$).

in batch II and 5.33 ± 2.03 IU/L in batch III treated animals. These results show a non-significant decrease in the activity of GGT in Lot II rats from 5.25 ± 1.49 (J0) to 2 ± 1 (J28). Rates of change in GGT activity in all treated animals ranging from 50% to 250% (J0); −20% to 86.66% (J14); −8.33% to 77.77% (J28) compared with the control Lot T.

3.3. Histological Study of Liver

Figure 8 shows histological sections of the liver in animals. These sections show an almost identical normal anatomical structure in the liver of rats from Lot T to Lot III. Hepatic cells show no alteration in tissue structure compared with controls.

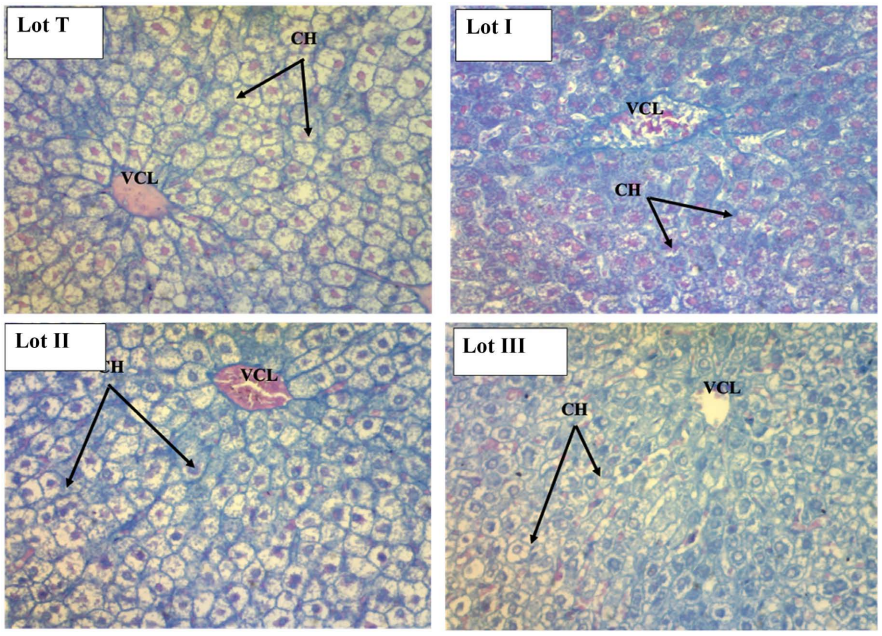

Figure 8. Portion of the liver of rats. Hemalun-eosin stain; magnification: ×100 T-lot (control): portion of control rat liver tissue; Lot I (200 mg/kg PC), Lot II (400 mg/kg PC), and Lot III (800 mg/kg PC); portions of liver tissue from rats treated at different doses. CH: Hepatic Cells, VCL: Centro-Lobular Vein.

4. Discussion

MTT assay demonstrates that mitochondrial activity in Vero cells is linked to cell proliferation. MTT is widely used as a measure of cytotoxicity and cell viability after exposure of living cells to toxic substances [17] [18]. MTT results show a decrease in the percentage viability of Vero cells exposed to Antipalu at incubation times of 24, 48 and 72 hours. This decrease is highly significant for concentrations of 250 mg/mL and 500 mg/mL, after 48 H of exposure to Antipalu. In parallel with mitochondrial activity by MTT assay, the 10% and 50% inhibitory concentrations (IC_{10} and IC50 respectively) were determined. Antipalu showed IC_{10} = 111 µg/mL and IC_{50} = 555 µg/mL). These results suggest that mitochondrial metabolism and/or cell proliferation are perturbed. These results align with the work of Badakhshan *et*

al., who demonstrated in their study on the cytotoxicity of *Lantana camara* leaf extract on the Vero cell line that the extract inhibited cell growth at concentrations up to 500 µg/mL [19].

Although the pathophysiological mechanisms remain poorly elucidated, the hypothesis of phytomedicinal toxicity has been addressed in numerous epidemiological studies. Indeed, several cytotoxicity tests have been carried out on medicinal plants, and the damage or loss of integrity of cell membranes leading to cell death directly results in the release of cytosolic enzymes such as LDH into the extracellular medium [20]. Also, cell death and/or membrane lysis correlate with the level of enzyme activity and provide an accurate measure of cytotoxicity induced by compounds contained in chemical substances [21].

Assays carried out on supernatants from Vero cells exposed to Antipalu extract showed an increase in extracellular LDH activity on the cells at different exposure times of 24, 48 and 72 hours. This increase was highly significant ($P < 0.001$) for concentrations between 3.9 mg/mL and 1000 µg/ml after 72 h exposure, indicating Antipalu's capacity to alter cell membranes. Our results are similar to those of (Abdallah *et al*., 2014) who showed a strong correlation between the use of *Mangifera indica* plant extracts and MTT and LDH assays (Abdallah *et al*., 2014). Overall, these results indicate that Antipalu recorded in Abidjan induced global cytotoxicity *in vitro* on exposed Vero cells.

Regarding the hepatic damage of Antipalu, the results showed that administration of Antipalu to the animals did not lead to variations in the hepatic biochemical markers ALAT, ASAT, ALPs and GGT. Indeed, ALAT, ASAT, ALPs and, to a lesser extent, GGT are enzymes frequently analyzed to assess liver damage [22]. Also, cell necrosis or deterioration of liver parenchyma or increased membrane permeability of hepatocytes releases enzymes into the bloodstream, thereby increasing hepatic serum levels [23] [24]. Measuring the activity of these enzymes is proportional to the degree of liver damage, making it a good indicator of hepatotoxicity [24].

Our results are similar to those found by Kone *et al.* on a traditional African phytomedicine, which showed that prolonged administration led to moderate elevations in AST and ALT without significant changes in cardiac markers [25]. This suggests that toxic effects may be more pronounced on other organs than on the liver.

Research also demonstrated in their work the absence of disruption of certain hepatic biochemical markers after administration of a phytomedicine to albino Wistar rats over a 28-day period, with doses of 3.5, 35 and 350 mg/kg body weight [25]. However, these results differ from those obtained by Gbogbo *et al.* 2014, who showed that subacute administration of the aqueous total extract of *Spondias mombin* L. stem barks resulted in a gradual increase in liver markers over the course of treatment [26].

5. Conclusion

This study focused on Antipalu, a phytomedicine used in the treatment of malaria.

The results indicate that Antipalu in Abidjan induced global cytotoxicity *in vitro* on Véro cells through cell death and/or membrane lysis. The work also revealed that exposure of Véro cells to Antipalu induced a decrease in mitochondrial activity in these Véro cells, with IC_{10} = 111 µg/mL and IC_{50} = 555 µg/mL. This study, which is the first to assess the toxicity of medicinal beverages on cell lines, merits further investigation in order to establish a better correlation between the daily intake of Antipalu and its potential adverse effects on the health of the aforementioned population. However, Antipalu did not affect hepatic parameters when administered at doses of 200, 400 and 800 mg/kg body weight over a 28-day period. In sum, the combined knowledge of cytotoxicity, chemical ethnopharmacology and other cardiac or renal markers is essential for a comprehensive assessment of Antipalu's potential health effects.

Acknowledgements

We express gratitude to the Department of Biochemistry of Université Félix Houphouët-Boigny of Abidjan (Ivory Coast), Institute Pasteur de Côte d'Ivoire, and the authorities of the Superior Normal School of Abidjan for providing the facilities in conducting this research.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] OMS (2013) Stratégie de l'OMS pour la médecine traditionnelle pour 2014-2023. Organisation mondiale de la Santé.

[2] Ouoba, K., Lehmann, H., Semde, R. and Pabst, J. (2022) Literature Review on Pharmacovigilance of Medicines Derived from Traditional Pharmacopoeias. Part I: Risks Identification. *Annales Pharmaceutiques Francaises*, **80**, 646-658. https://doi.org/10.1016/j.pharma.2021.11.008

[3] Najjaa, H., Zouari, S., Arnault, I., Auger, J., Ammar, E. and Neffati, M. (2011) Diffrences et similitudes des métabolites secondaires chez deux espèces du genre Allium, *Allium roseum* L. *et Allium ampeloprasum* L. *Acta Botanica Gallica*, **158**, 111-123. https://doi.org/10.1080/12538078.2011.10516259

[4] WOH (2004) WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems. World Health Organization.

[5] Adjoungoua, A., Diafouka, F., Koffi, P., Lokrou, A. and Attaï, H. (2006) Valorisation de la pharmacopée traditionnelle: Action de l'extrait alcoolique de Bidens pilosa (Asteraceae) sur l'exploration statique et dynamique de la glycémie. *Revue de Médecines et Pharmacopées Africaines*, **19**, 1-12.

[6] Tra-Bi, I.O., Djyh, B.N., Bahi, C., Meite, S. and N'guessan, J.D. (2011) A Study of the Hepatic Tolerance of the Ethyl Acetate Fraction of *Morinda morindoides* (Baker) *Milne-Redhead* (Rubiaceae) Extract in Rabbit. *Tropical Journal of Pharmaceutical Research*, **9**, 573-579. https://doi.org/10.4314/tjpr.v9i6.63582

[7] Debelle, F.D., Vanherweghem, J. and Nortier, J.L. (2008) Aristolochic Acid Nephropathy: A Worldwide Problem. *Kidney International*, **74**, 158-169. https://doi.org/10.1038/ki.2008.129

[8] Rivière, C., Nicolas, J., Caradec, M., Désiré, O. and Schmitt, A. (2005) Les plantes médicinales de la région nord de Madagascar: Une approche ethnopharmacologique. *Bulletin de la Société Française d'Ethnopharmacologie*, **36**, 36-49.

[9] OCDE (2008) Essai n° 407: Toxicité orale à doses répétées—Pendant 28 jours sur les rongeurs.

[10] OCDE (2002) Test No. 423: Acute Oral Toxicity—Acute Toxic Class Method.

[11] Kamo, I.L.B.E., Kouassi, K.S., Atto, V., Djaman, A.J., N'guessan, J.D. and Dosso, M. (2024) Cardiac Tolerance of Hydroalcoholic Extract of Bark of *Terminalia mantaly* H. Perrier (HAEBTM) in Wistar Rats. *American Journal of Molecular Biology*, **14**, 126-137. https://doi.org/10.4236/ajmb.2024.143010

[12] Jamour, P., Jamali, A., Langeroudi, A.G., Sharafabad, B.E. and Abdoli, A. (2024) Comparing Chemical Transfection, Electroporation, and Lentiviral Vector Transduction to Achieve Optimal Transfection Conditions in the Vero Cell Line. *BMC Molecular and Cell Biology*, **25**, Article No. 15. https://doi.org/10.1186/s12860-024-00511-x

[13] Sène, M., Xia, Y. and Kamen, A.A. (2022) Overview of Recent Advances in Vero Cells Genomic Characterization and Engineering for High-Throughput Vaccine Manufacturing. *Clinical and Translational Discovery*, **2**, e40. https://doi.org/10.1002/ctd2.40

[14] Emilie, K.I.L.B., Otis, T.B.I., Serge, K.K., Joseph, D.A. and David, N.J. (2022) Biotolerance Study of the Hydroalcoholic Extract of *Terminalia mantaly* H. Perrier on Rat Renal Activity. *Journal of Biosciences and Medicines*, **10**, 167-179. https://doi.org/10.4236/jbm.2022.1012014

[15] Osifo, U., Akpamu, U., Idehen, I., Adisa, A. and Azeke, E. (2012) The Effect of Chronic Ingestion of Crude Garcinia Kola on the Histology of the Liver. *European Journal of Experimental Biology*, **2**, 404-409.

[16] Koudou, D., Otis, T.-B.I., Oussou, J., Mama, K., Yapo, A. and Djaman, A. (2017) Cardiac Tolerance of an Ethyl Acetate Extract of *Holarrhena floribunda* (G. Don) Durand and Schinz Leaves in Wistar Rats. *Scholars Academic Journal of Pharmacy*, **6**, 229-235.

[17] Bernhard, D., Schwaiger, W., Crazzolara, R., Tinhofer, I., Kofler, R. and Csordas, A. (2003) Enhanced MTT-Reducing Activity under Growth Inhibition by Resveratrol in CEM-C7H2 Lymphocytic Leukemia Cells. *Cancer Letters*, **195**, 193-199. https://doi.org/10.1016/s0304-3835(03)00157-5

[18] Mosmann, T. (1983) Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *Journal of Immunological Methods*, **65**, 55-63. https://doi.org/10.1016/0022-1759(83)90303-4

[19] Pour, B.M., Latha, L.Y. and Sasidharan, S. (2011) Cytotoxicity and Oral Acute Toxicity Studies of *Lantana Camara* Leaf Extract. *Molecules*, **16**, 3663-3674. https://doi.org/10.3390/molecules16053663

[20] Specian, A.F.L., Serpeloni, J.M., Tuttis, K., Ribeiro, D.L., Cilião, H.L., Varanda, E.A., *et al*. (2016) LDH, Proliferation Curves and Cell Cycle Analysis Are the Most Suitable Assays to Identify and Characterize New Phytotherapeutic Compounds. *Cytotechnology*, **68**, 2729-2744. https://doi.org/10.1007/s10616-016-9998-6

[21] Korzeniewski, C. and Callewaert, D.M. (1983) An Enzyme-Release Assay for Natural Cytotoxicity. *Journal of Immunological Methods*, **64**, 313-320. https://doi.org/10.1016/0022-1759(83)90438-6

[22] Dillon, J.F. and Miller, M.H. (2016) Gamma Glutamyl Transferase "to Be or Not to Be" a Liver Function Test? *Annals of Clinical Biochemistry*: *International Journal of*

Laboratory Medicine, **53**, 629-631. https://doi.org/10.1177/0004563216659887

[23] Manda, P., Manda, O.M., Manda, V., Kroa, E. and Dano, S.D. (2017) Etude des toxicités aigue et subaiguë du remede nature utilise dans le traitement du paludisme. *Revue Ivoirienne des Sciences et Technologie*, **29**, 145-458.

[24] Pariente, A. (2013) Cytolyse hepatique (augmentation des aminotransferases) chez l'adulte. *Hépato-Gastro & Oncologie Digestive*, **20**, 629-638.

[25] Kone, M., Bleyere, N., Yapo, A., Vangah, M. and Ehile, E. (2010) Evaluation de la toxicité d'un extrait aqueux de *Sacoglottis gabonensis* (Baille) Urban (Humiriaceae) chez les rongeurs, une plante utilisée dans le traitement de l'ulcère de Buruli en Côte d'Ivoire. *International Journal of Biological and Chemical Sciences*, **3**, 1286-1296. https://doi.org/10.4314/ijbcs.v3i6.53147

[26] Moussa, G., Mama, K., Mathieu, B.N., Emeli, Y. and Paul, Y.A. (2014) Effect of Total Aqueous Stem Bark Extract of *Spondias mombin* L. on Some Biochemical and Anthropometric Parameters in Wistar Albino Rats. *International Journal of Biosciences*, **4**, 1-8.