Scientific Research Publishing

American Journal of Plant Sciences, 2025, 16(9), 1083-1097
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Study of the Acute Oral Toxicity, 28-Day Subchronic Toxicity, and Cytotoxicity of a Herbal Antiviral Recipe Used in the Treatment of Hepatitis B in Togo

Minyo Ega Sossa Montant[1], Bouraïma Djeri[1], Kosi Mawuéna Novidzro[2], Ablam Alognon[1], Sossawè Poli[1*], Yao Hoinsou[1], Luckman Gbati[1], Gérard Toudji[1], Tibanguebe Doumongue[3], Fo-Doh Clefasse Koula[3], Kossi Kabo[3], Kofivi Mawouko Yena[4], Efui Holaly Gbekley[1,3,5,6], Damintoti Simplice Karou[1]

[1]Microbiology and Food Quality Control Laboratory (LAMICODA), Higher School of Biological and Food Techniques (ESTBA), University of Lomé, Lomé, Togo
[2]Laboratory of Engineering of Processes and Natural Resources (LAGEPREN) of the University of Lomé, Lomé, Togo
[3]Laboratory of Biomedical Sciences, Food and Environmental Health—Research Unit in Biomedical Sciences and Bioactive Substances (LaSBASE-UR-2SB), Higher School of Biological and Food Techniques (ESTBA), University of Lomé, Lomé, Togo
[4]University of Lorraine, Inserm CIC P1433 Multidisciplinary Clinical Investigation Center, Inserm U1116, CHRU Nancy Brabois, F-CRIN INI-CRCT (Cardiovascular and Renal Clinical Trialists), Nancy, France
[5]Department of Biochemistry/Nutrition, Laboratory of Biochemistry Applied to Nutrition, Faculty of Sciences, University of Lomé, Lomé, Togo
[6]Laboratory of Biology, Phytochemistry, Toxicology, Pharmacology and Agrifood (BioPhytToPharmA), Institute African Biomedical, Agrifood, Societal and Environmental Sciences (IASBASE), Lomé, Togo
Email: *sosinhopoli@gmail.com

How to cite this paper: Montant, M.E.S., Djeri, B., Novidzro, K.M., Alognon, A., Poli, S., Hoinsou, Y., Gbati, L., Toudji, G., Doumongue, T., Koula, F.-D.C., Kabo, K., Yena, K.M., Gbekley, E.H. and Karou, D.S. (2025) Study of the Acute Oral Toxicity, 28-Day Subchronic Toxicity, and Cytotoxicity of a Herbal Antiviral Recipe Used in the Treatment of Hepatitis B in Togo. *American Journal of Plant Sciences*, **16**, 1083-1097.
https://doi.org/10.4236/ajps.2025.169070

Received: April 27, 2025
Accepted: September 15, 2025
Published: September 18, 2025

Abstract

This study was conducted to evaluate the safety of a hydroethanolic extract from a herbal recipe composed of *Bridelia ferruginea* bark, *Sansevieria liberica* roots, and the whole plant of *Phyllanthus amarus*, which is traditionally used in the treatment of hepatitis B in Togo. Acute and subchronic toxicity tests were conducted following OECD guidelines 423 and 407, respectively. For the acute toxicity study, a single dose of 5000 mg/kg body weight (bw) of the recipe extract was administered. For the subchronic toxicity study, doses of 500 and 1000 mg/kg bw were used. Cytotoxicity was evaluated using the MTS assay, and normal human colon epithelial cells (NCM 356) were used. In the acute toxicity study, the recipe extract caused no deaths or signs of toxicity. In the subchronic study, administration of 500 and 1000 mg/kg bw over 28 consecutive days in Wistar rats caused no mortality. No macroscopic lesions attributable to treatment were observed in either male or female rats. At a dose

of 500 mg/kg bw, the extract significantly reduced the relative weight of the testes ($p = 0.013$) and liver ($p < 0.001$) in male rats. In female rats, a significant increase in creatinine was observed at 500 mg/kg ($p = 0.0083$), and alanine aminotransferase levels increased significantly at 1000 mg/kg bw ($p = 0.002$) compared to the control group. The extract showed no cytotoxicity in the MTS assay.

Keywords

Acute Toxicity, Subchronic Toxicity, Cytotoxicity, *Bridelia ferruginea*, *Sansevieria liberica*, *Phyllanthus amarus*

1. Introduction

Hepatitis B is a chronic viral disease with a high epidemic potential and remains a major public health concern in sub-Saharan Africa [1]. According to the World Health Organization (WHO), nearly 82 million people in the region are living with chronic hepatitis B virus (HBV) infection, representing about 25% of all global cases [1]. Togo is among the countries with the highest prevalence, with rates estimated between 10% and 14%, depending on the population group studied [2]. Chronic HBV infection can lead to severe health complications, including liver fibrosis, cirrhosis, and hepatocellular carcinoma [3].

In a context marked by limited resources and reduced access to standard antiviral treatments (such as tenofovir, disoproxil, fumarate), many people in Togo turn to traditional medicine for care. Herbal remedies, often passed down through generations, are widely used to relieve symptoms linked to liver diseases, including jaundice, liver pain, and digestive issues [4] [5]. Some of these plants based formulations, typically made from a mix of several species, are specifically recommended for their supposed antiviral activity against HBV. However, scientific evidence regarding their effectiveness and safety remains scarce.

Unlocking the potential of these local therapeutic resources calls for a rigorous toxicological evaluation to ensure their safe use. While medicinal plants are natural, they may contain active secondary metabolites (such as alkaloids, saponins, and tannins) that can cause adverse effects, especially in vital organs like the liver, kidneys, or hematopoietic system [6]. These toxic effects may be either acute or chronic and could accumulate over time, especially with prolonged use or interactions between different plant components [6].

Given this context, evaluating both the acute and 28-day subchronic oral toxicity of a locally used herbal preparation for hepatitis B treatment, along with its cytotoxicity on human cell lines, is essential to promote its safe and informed use. This type of investigation is in line with WHO recommendations and OECD guidelines concerning the safety evaluation of traditional medicinal products.

The objectives of this study are therefore to:

- Determine the oral median lethal dose (LD_{50}) of the recipe in rodents;
- Evaluate biological effect after repeated 28-day administration (subchronic

toxicity);

- Assess in vitro cytotoxicity on human liver cell lines.

The results of this research will contribute to establishing a preliminary toxicological profile for the herbal formulation, providing a foundation for future pharmacological and clinical studies.

2. Materials and Methods

2.1. Study Framework

This research was carried out at multiple institutions. Laboratory work was conducted at the Microbiology and Food Quality Control Laboratory of the Faculty of Food Science and Technology (ESTBA), University of Lomé, and at the Regional Center of Excellence in Poultry Sciences, both located in Togo. Additional analyses were performed at the Biomedical Research Centre of the University of Granada (Granada, Spain).

2.2. Chemicals and Reagents

Various chemicals and reagents such as DNEM (Dulbecco's Modified Eagle Medium, FBS (Foetal calf serum), 3-(4,5-dimethylthiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, Ether were procured from SIGMA (USA).

2.3. Plant Material

The herbal preparation used in this study was composed of bark from *Bridelia ferruginea*, roots of *Sansevieria liberica*, and the whole plant of *Phyllanthus amarus*. The plant materials were collected in June 2023 from Hahotoé, a village located northeast of Lomé, Togo. Botanical identification and authentication were performed at the Laboratory of Botany and Plant Ecology, Faculty of Sciences, University of Lomé. The following voucher specimens were deposited: *Sansevieria liberica* Hort. ex Gérôme & Labroy (TG201529), *Bridelia ferruginea* Benth. (7382 FDS/UL), and *Phyllanthus amarus* Schumach. & Thonn (TG201530).

2.4. Animals

The animals used in this study were obtained from the Laboratory of Microbiology and Food Quality Control (LAMICODA) of ESTBA. All animal procedures were approved by the Ethics Committee for Animal Experimentation of ESTBA, University of Lomé, in accordance with international guidelines (ARRIVE and Directive 2010/63/EU). The number of animals used was minimized to ensure both ethical compliance and scientific validity.

Healthy female Wistar albino rats, weighing between 100 and 120 g and free from any visible signs of illness or injury, were used for the acute and subchronic toxicity evaluations. The animals were fed wheatgrass and had free access to water. They were housed in standard laboratory conditions: temperature 26˚C ± 4˚C, relative humidity around 60%, and a 12-hour light/dark cycle. All experimental

procedures were conducted between April and May 2024.

2.5. Extraction

The collected plant parts were shade-dried at the Laboratory of Microbiology and Food Quality Control (LAMICODA), University of Lomé, at ambient temperatures ranging from 26˚C to 30˚C. Once fully dried, the materials were finely ground into powder. Equal amounts (100 g) of powder from each plant part were mixed and macerated in 3 liters of a 70:30 ethanol-water solution. The mixture was left to macerate for 72 hours, with gentle stirring every 24 hours. After maceration, the mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated at 45˚C using a Heidolph rotary evaporator, then frozen at −30˚C and lyophilized at −45˚C. The resulting dry extract was stored in airtight, opaque containers at a refrigerated temperature between 4˚C and 8˚C until use.

2.6. Acute Toxicity Study

The experiment followed OECD guideline 423 for the testing of chemicals [7].

A single-limit dose of 5000 mg/kg body weight of the plant extract was administered to assess its acute toxicity. Two groups of three healthy female Wistar rats were formed: the test group received the extract, while the control group was given physiological water. Food was withheld for 12 hours prior to administration, although water was available ad libitum. Rats were weighed immediately before dosing, and the extract was administered orally via gavage following a sequential dosing procedure.

Following administration, animals were observed individually during the first 30 minutes, continuously during the first 24 hours and then once daily for a total of 14 days. Observations focused on general behavior and clinical signs of toxicity, including changes in skin and fur, locomotor activity, posture, and any symptoms such as tremors, convulsions, salivation, diarrhea, lethargy, sleep, or coma. Body weight was recorded daily throughout the 14-day observation period.

2.7. Subchronic Toxicity Study

The 28-day oral subchronic toxicity of the herbal extract was assessed in both male and female Wistar rats, in accordance with OECD Guideline 407 for the testing of chemicals [8].

Three groups of 10 rats each (5 males and 5 females), weighing between 100 and 120 g, were used for the study. Group 1 served as the control and received physiological water. Group 2 and Group 3 received daily oral doses of the herbal extract at 500 mg/kg body weight and 1000 mg/kg body weight, respectively. Dosing was carried out by oral gavage using an esophageal probe, administered at the same time each day for 28 consecutive days.

Animals were observed at least twice daily for signs of morbidity or mortality. Body weight was measured daily prior to dosing to monitor changes over the treatment period.

At the end of the 28-day treatment, animals were fasted for 12 hours, anesthe-

tized with ether, and blood samples were collected via retro-orbital puncture. Blood was drawn into dry tubes for biochemical analyses (including urea, glucose, creatinine, ALT, AST, GGT, total cholesterol, and triglycerides) and into EDTA tubes for complete blood count (CBC). Following blood collection, the animals were euthanized, and a full necropsy was performed. Organs, including the heart, lungs, liver, kidneys, spleen, testes, and ovaries, were carefully excised, weighed, and used to calculate relative organ-to-body weight ratios.

General Behavior and Mortality: Before treatment began, all animals were individually handled and examined to detect any pre-existing abnormalities in behavior or appearance. Throughout the study, animals were monitored twice daily for signs of illness, behavioral changes, abnormal posture, or alterations in skin, fur, eyes, or mucous membranes. Gait and locomotor activity were assessed weekly by allowing the animals to move freely in an observation area.

Hematological and Biochemical Parameters: Blood samples collected in dry tubes were centrifuged at 3000 rpm for 15 minutes using a CENTRIFUGE 80-3. The resulting serum was transferred into cryotubes and analyzed using a MINDRAY BS-280 chemistry analyzer for biochemical markers. Hematological parameters: red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT) were measured from EDTA-treated blood using a MINDRAY BC-10 automatic hematology analyzer.

2.8. Cytotoxicity Analysis

Cytotoxicity was evaluated using the MTS assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA) [9].

Normal human colon epithelial cells (NCM 356) were obtained from the cell culture unit of the Centro de Instrumentación Científica (CIC) at the University of Granada, Spain. Cells were cultured in DMEM medium supplemented with 10% FBS, 2 mmol/L L-glutamine, 100 U/mL penicillin, and 1 mg/mL streptomycin, maintained at 37˚C in a humidified 5% CO_2 incubator.

A total of 3×10^4 NCM 356 cells were seeded per well in 96-well plates and allowed to adhere for 24 hours. Cells were then treated with the extract at concentrations of 2, 5, 10, 50, and 100 µg/mL, or with control medium, for 48 hours. The medium was then replaced with 90 µL/well of fresh medium plus 10 µL/well of MTS solution and incubated for 3 - 4 hours at 37˚C with 5% CO_2. Absorbance was measured at 490 nm using a plate reader. The assay was repeated three times (n = 3).

2.9. Statistical Analysis

All results are expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to identify significant differences. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. Data analysis was

carried out using R software, version 4.2.3.

3. Results

3.1. Acute Oral Toxicity Study

Throughout the acute toxicity study, no mortality or clinical signs of toxicity were observed in the treated animals. The rats maintained normal behavior, with no alterations in responsiveness to pain, noise, or movement. Furthermore, macroscopic examination of internal organs revealed no visible abnormalities, suggesting that the extract did not induce acute toxic effects at the administered dose.

3.2. 28 Days Subchronic Oral Toxicity Study

Over the 28-day treatment period, no deaths or overt signs of toxicity were recorded in any of the experimental groups, regardless of the dose administered (500 or 1000 mg/kg body weight). All animals appeared healthy and displayed normal behavior, with no notable differences in food or water intake between the control and treatment groups.

Body Weight

In female rats, both doses of the extract (500 and 1000 mg/kg bw) led to a statistically significant increase in body weight compared to controls. This increase was observed from day 2 to day 11 ($p < 0.0001$), and continued significantly from day 12 to day 19 ($p < 0.001$) (Figure 1).

In male rats, no significant body weight change was observed at the 500 mg/kg dose. However, rats receiving 1000 mg/kg showed a sustained and significant increase in body weight throughout the study period, from day 2 to day 28 ($p < 0.0001$), as illustrated in Figure 1.

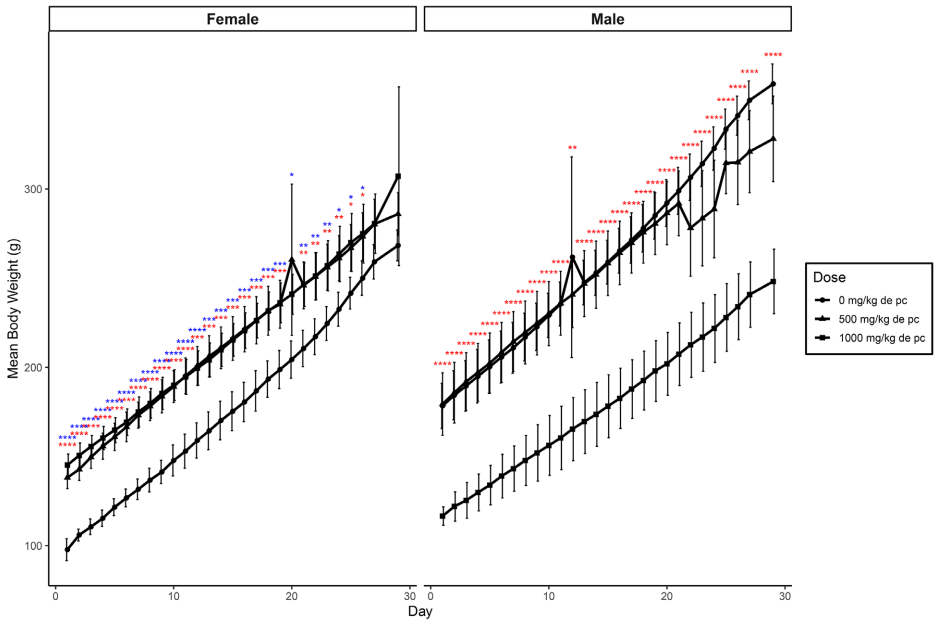

Figure 1. Evolution of rat body weight over 28 days of treatment with the hydroethanolic extract.

Relative Organ Weights and Macroscopic Observations: No treatment related macroscopic abnormalities were observed in any of the animals, regardless of sex or dosage level (500 and 1000 mg/kg bw). All internal organs appeared normal upon visual inspection during necropsy.

In female rats, there were no statistically significant differences in the relative weights of key organs, including the liver, spleen, heart, lungs, kidneys, and ovaries when compared to the control group, indicating that the extract did not cause any overt organ specific toxicity (Figure 2).

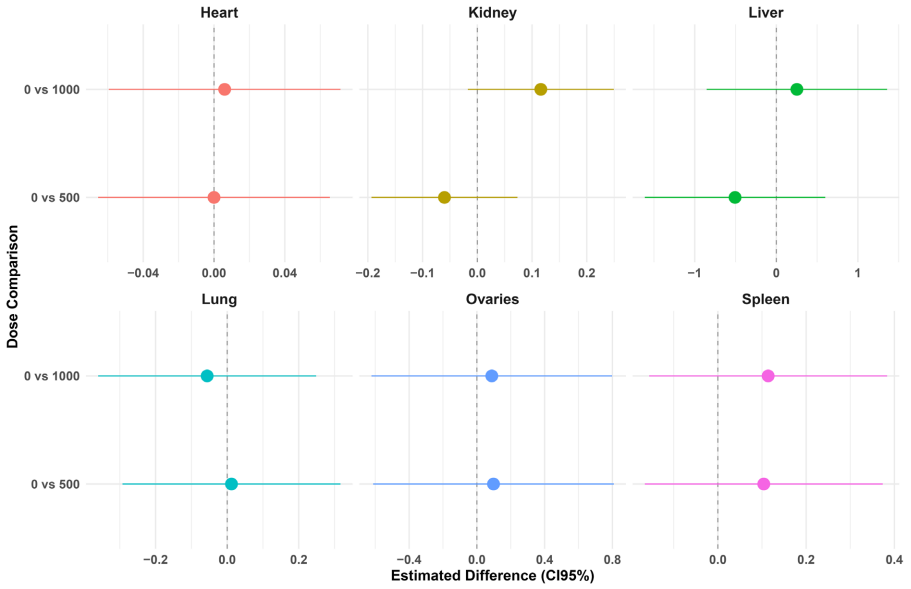

Figure 2. Estimated differences in mean organ weights in female rats with confidence intervals (95% CI).

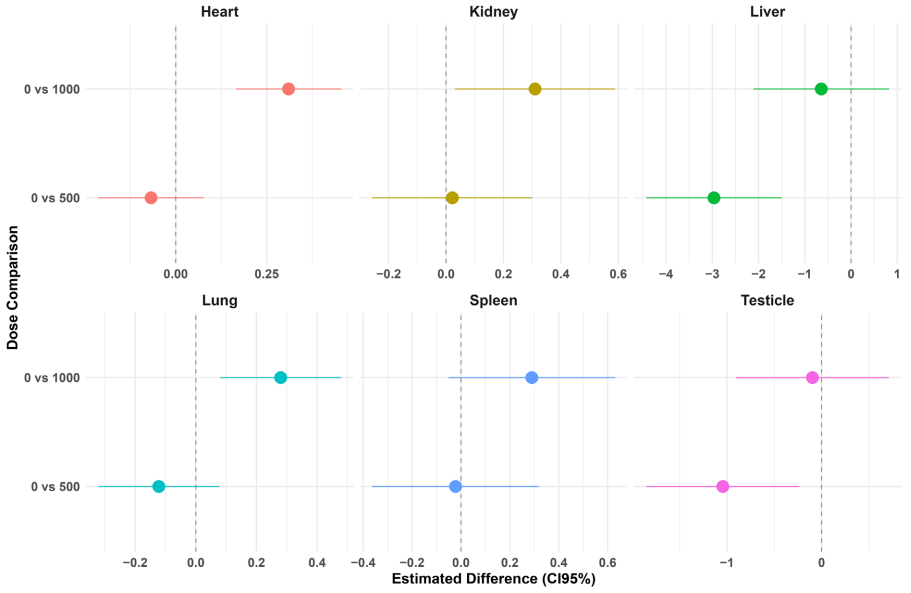

Figure 3. Estimated differences in mean organ weights in male rats with confidence intervals (95% CI).

At 500 mg/kg bw, a significant decrease in the relative weight of the testes ($p = 0.013$) and liver ($p < 0.001$) was observed in male rats. Conversely, at 1000 mg/kg bw, a significant increase in the relative weight of the lungs ($p = 0.0073$), kidneys ($p = 0.03$), and heart ($p > 0.01$) was noted (Figure 3).

Blood Biochemical Parameters: The serum biochemical profiles of female and male rats following 28 days of treatment are summarized in Table 1 and Table 2, respectively.

Table 1. Biochemical Parameters of female rats after 28 Days of Treatment.

Parameters	Control	Dose 500 mg/kg p.c.	Dose 1000 mg/kg p.c.
ALAT (U/L)	61.20 ± 14.45	47.40 ± 8.50	46.00 ± 11.68
ASAT (U/L)	233.80 ± 24.57	167.80 ± 34.41	143.40 ± 11.84
Creatinine (mg/L)	9.00 ± 1.00	9.00 ± 1.52	8.40 ± 0.89
Urea (g/L)	0.56 ± 0.05	0.36 ± 0.02	0.34 ± 0.06
Glycemia (g/L)	0.77 ± 0.07	0.92 ± 0.10	0.80 ± 0.10
Cholesterol (g/L)	0.44 ± 0.03	0.42 ± 0.02	0.56 ± 0.04
Triglycerides (g/L)	0.47 ± 0.05	0.50 ± 0.05	0.53 ± 0.03
Gamma GT (g/L)	19.20 ± 2.28	14.60 ± 2.19	16.60 ± 2.41

Values are expressed as mean ± standard deviation (SD).

Table 2. Biochemical Parameters of male rats after 28 Days of Treatment.

Parameters	Control	Dose 500 mg/kg p.c.	Dose 1000 mg/kg p.c.
ALAT (U/L)	43.20 ± 2.77	46.80 ± 16.99	64.40 ± 11.39
ASAT (U/L)	170.60 ± 5.90	201.80 ± 4.02	142.60 ± 31.34
Creatinine (mg/L)	8.40 ± 0.55	10.20 ± 0.83	8.50 ± 0.89
Urea (g/L)	0.37 ± 0.01	0.38 ± 0.03	0.43 ± 0.09
Glycemia (g/L)	0.68 ± 0.06	0.82 ± 0.12	0.87 ± 0.16
Cholesterol (g/L)	0.67 ± 0.04	0.42 ± 0.03	0.67 ± 0.44
Triglycerides (g/L)	0.45 ± 0.06	0.45 ± 0.04	0.51 ± 0.05
Gamma GT (g/L)	20.20 ± 1.92	16.60 ± 2.70	16.60 ± 2.97

Values are expressed as mean ± standard deviation (SD).

In female rats, no significant changes were observed in urea, glucose, AST, GGT, total cholesterol, or triglycerides compared to controls. However, a significant increase in creatinine was observed at 500 mg/kg bw ($p = 0.0083$), and ALT increased significantly at 1000 mg/kg bw ($p = 0.002$) (Figure 4).

In male rats, no significant changes in glucose, creatinine, ALT, or triglycerides were observed. However, AST was significantly reduced at both 500 mg/kg ($p < 0.01$) and 1000 mg/kg ($p < 0.001$). GGT also decreased at 500 mg/kg ($p = 0.02$), as did total cholesterol at 1000 mg/kg ($p < 0.001$) and urea at both doses ($p < 0.001$) (Figure 5).

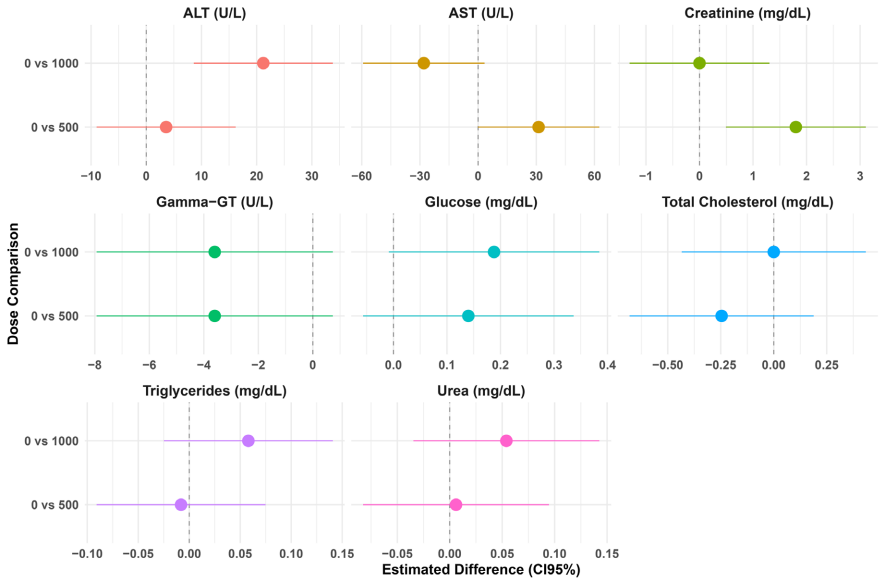

Figure 4. Estimated differences in biochemical data of female rats with 95% confidence intervals (95% CI).

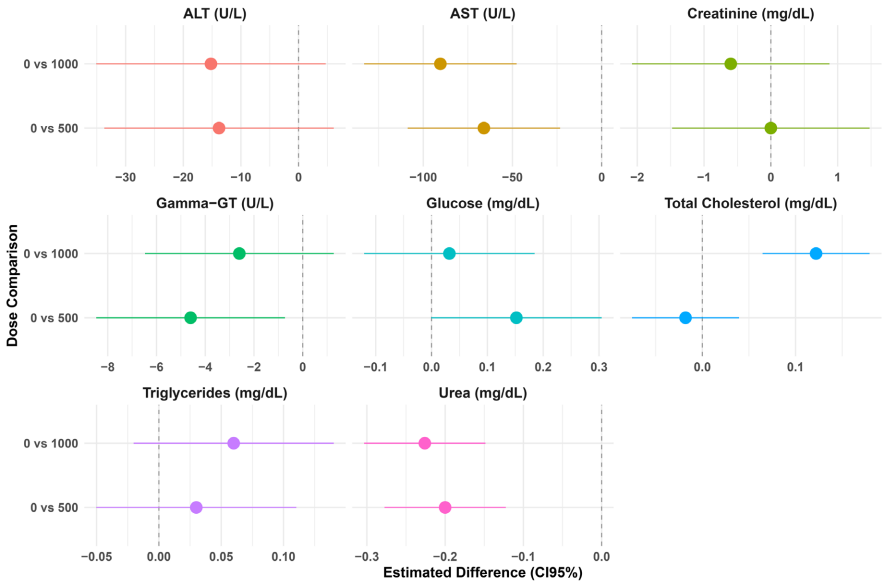

Figure 5. Estimated differences in biochemical data of male rats with 95% confidence intervals (95% CI).

Hematological Parameters

Hematological data are presented in Table 3 and Table 4.

Table 3. Hematological parameters of male rats after 28 days of treatment.

Parameters	Physiological water	Dose 500 mg/kg p.c.	Dose 1000 mg/kg p.c.
		Male rats	
WBC (10^3/µL)	8.36 ± 0.65	7.35 ± 1.90	9.60 ± 0.25
RBC (10^6/µL)	7.38 ± 0.47	7.35 ± 0.34	8.01 ± 1.00

Continued

HGB (g/dL)	13.18 ± 0.70	12.29 ± 0.61	13.36 ± 0.72
HCT (%)	38.04 ± 2.46	36.62 ± 0.99	40.74 ± 1.59
MCV (fl)	51.48 ± 1.12	49.12 ± 1.95	48.74 ± 1.99
MCH	17.74 ± 0.73	16.58 ± 0.82	16.04 ± 0.46
MCHC (g/dL)	34.84 ± 1.05	33.58 ± 1.01	32.96 ± 0.81
PLT (10^3/µL)	764.2 ± 105.48	790.20 ± 185.60	770.80 ± 55.40

Values are expressed as mean ± standard deviation (SD).

Table 4. Hematological parameters of female rats after 28 days of treatment.

Parameters	Physiological water	Dose 500 mg/kg p.c.	Dose 1000 mg/kg p.c.
	Female rats		
WBC (10^3/µL)	6.86 ± 1.44	6.54 ± 0.42	8.32 ± 2.23
RBC (10^6/µL)	7.22 ± 0.27	6.85 ± 0.63	6.80 ± 0.63
HGB (g/dL)	13.32 ± 0.34	12.64 ± 1.04	12.68 ± 0.46
HCT (%)	39.80 ± 1.59	34.64 ±1.64	36.80 ± 1.55
MCV (fl)	53.86 ± 1.72	53.88 ± 1.87	52.96 ± 2.69
MCH	18.28 ± 0.35	18.16 ± 0.54	18.36 ± 1.38
MCHC (g/dL)	34.16 ± 0.65	34.02 ± 1.45	34.56 ± 0.92
PLT (10^3/µL)	769.6 ± 245.76	821.80 ± 96.39	754.00 ± 79.70

Values are expressed as mean ± standard deviation (SD).

In female rats, a significant decrease in hematocrit was observed at both 500 mg/kg ($p < 0.001$) and 1000 mg/kg ($p = 0.029$) compared to the control group. No significant differences were found in other hematological parameters including hemoglobin (HGB), MCV, MCH, MCHC, or platelet count (PLT) (Figure 6).

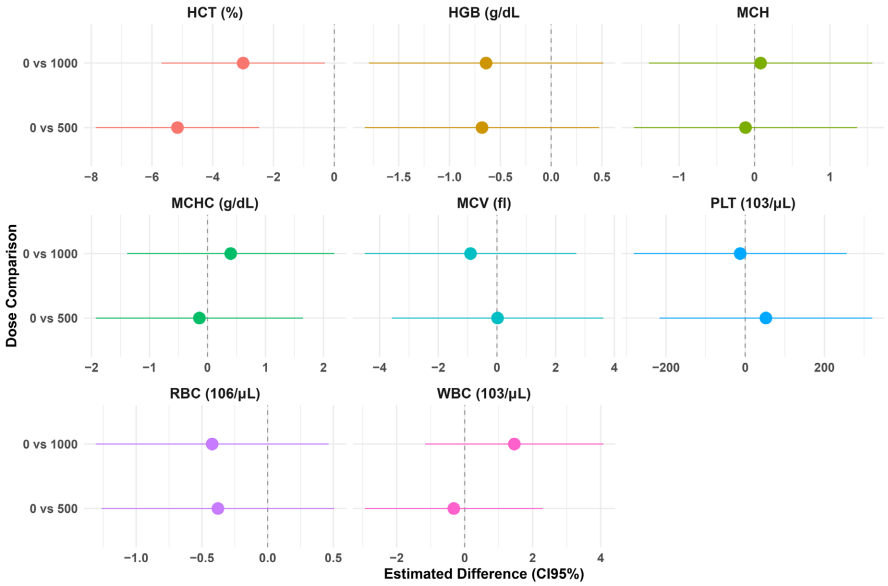

Figure 6. Estimated differences in hematological data of female rats with 95% confidence intervals (95% CI).

In male rats treated with 500 and 1000 mg/kg, no statistically significant changes were observed in RBC, HGB, HCT, MCV, WBC, or PLT. However, MCH levels showed a significant reduction at 500 mg/kg ($p < 0.01$) and 1000 mg/kg ($p < 0.001$), while MCHC significantly decreased at 1000 mg/kg ($p = 0.024$) (Figure 7).

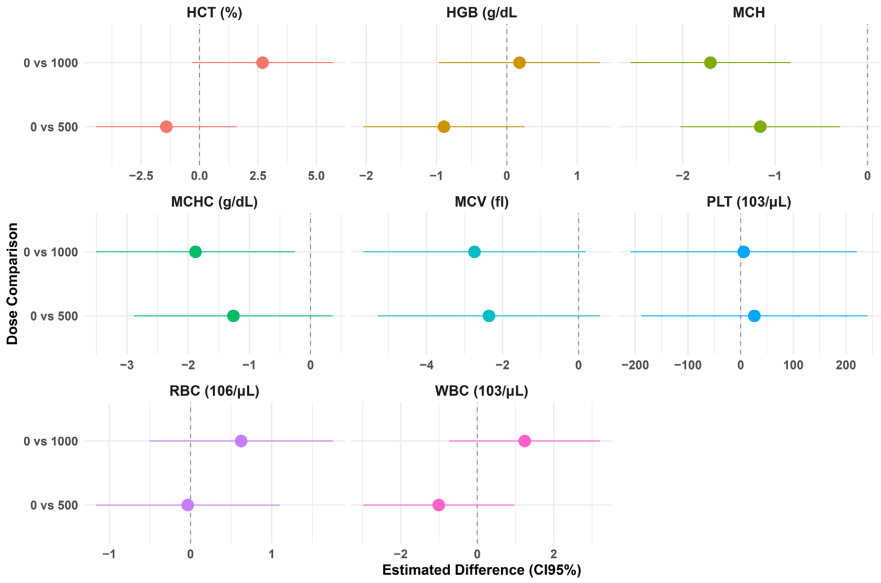

Figure 7. Estimated differences in hematological data of male rats with 95% confidence intervals (95% CI).

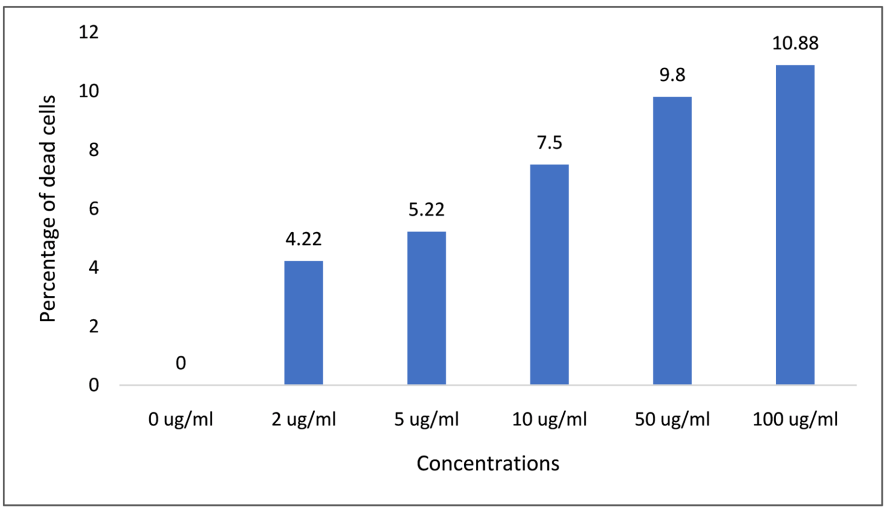

Figure 8. Cell death based on concentrations.

3.3. Cytotoxicity Evaluation

Cytotoxicity was evaluated using the MTS assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA) [9]. Normal human colon epithelial cells (NCM 356) were obtained from the cell culture unit of the Centro de Instrumentación Científica (CIC) at the University of Granada, Spain. Cells were cultured in DMEM medium supplemented with 10% FBS, 2 mmol/L

L-glutamine, 100 U/mL penicillin, and 1 mg/mL streptomycin, maintained at 37˚C in a humidified 5% CO_2 incubator.

Cytotoxicity was recorded as CC_{50} values (mg/mL). This corresponds to the extract concentration that kills or inhibits the growth of 50% of the cell population. The percentage of cell lethality at the highest concentration was 10.88% (**Figure 8**). The obtained data show that the extract from the recipe does not have the potential to inhibit the growth of NCM in a time- and dose-dependent manner. The cytotoxic concentration for 50% of the cells (CC_{50}) is 440,457 µg/mL, equivalent to 440.46 mg/mL.

4. Discussion

This study focused on evaluating the acute, subchronic, and cytotoxic oral toxicity of a hydroethanolic extract from an herbal antiviral recipe composed of *Bridelia ferruginea* bark, *Sansevieria liberica* roots, and the entire *Phyllanthus amarus* plant, traditionally used in the treatment of hepatitis B in Togo. While previous studies have explored the individual toxicological profiles of these plants, no prior research has evaluated the safety of their combined use in this specific formulation, despite its known antiviral properties. In our acute oral toxicity study, no deaths or signs of toxicity such as reduced sensitivity to pain, noise, or locomotion were observed in rats. These findings suggest that the median lethal dose (LD_{50}) of the hydroethanolic extract exceeds 5000 mg/kg body weight when administered orally. The findings of Hoinsou *et al.* (2024) are consistent with our results. In our study, no deaths or signs of toxicity were observed in rats following oral administration of the hydroethanolic extract formulated from three medicinal plants traditionally used to treat typhoid fever in the Maritime region of Togo. The extract showed an LD_{50} greater than 5000 mg/kg body weight [10]. According to the Globally Harmonized System (GHS) for the classification and labeling of chemicals, a compound is not considered toxic, harmful, or lethal if the LD_{50} exceeds 5000 mg/kg [11]. Similar results were reported in prior studies on the acute toxicity of the individual components of the recipe. For example, Bakoma *et al.* (2013) showed an LD_{50} greater than 5000 mg/kg for the hydroethanolic extract of *Bridelia ferruginea* bark [12]. Likewise, Lawson-Evi *et al.* (2014) demonstrated that a single oral dose of 5 g/kg of *Phyllanthus amarus* extract caused no mortality or significant change in animals over a 14-day observation period [13].

In the subchronic toxicity study, no mortality or clinical signs of toxicity were observed in rats treated with the extract for 28 consecutive days at doses of 500 or 1000 mg/kg body weight. Relative organ weight is a key indicator of physiological and pathological changes in toxicological evaluations [14] as it can help detect early signs of organ damage or dysfunction [15]. In this study, the extract did not induce any significant alterations in organ-to-body weight ratios, nor were there any visible morphological changes in the examined organs. The liver, being central to nutrient metabolism, protein synthesis, energy production, and drug detoxification, is often a primary focus in toxicity assessments [16]. Its functional

integrity is typically monitored by measuring serum levels of enzymes such as ALT, AST, ALP, and GGT. Elevations in ALT and AST levels are commonly associated with hepatocellular injury [17]. However, a significant decrease in AST levels was observed at both 500 mg/kg ($p < 0.01$) and 1000 mg/kg ($p < 0.001$). GGT levels also decreased significantly at 500 mg/kg ($p = 0.02$), while total cholesterol showed a marked reduction at 1000 mg/kg ($p < 0.001$). In addition, urea levels were significantly reduced at both doses ($p < 0.001$). These findings are in line with previous studies by Oduola *et al.* (2018), Bakoma *et al.* (2013), and Amida *et al.* (2017), which found no significant changes in ALT, AST, GGT, creatinine, or urea levels after administering extracts of *Phyllanthus amarus*, *Bridelia ferruginea*, or *Sansevieria liberica*, respectively [17]-[19]. Overall, these results suggest that the hydroethanolic extract of the herbal recipe did not negatively impact liver or kidney function in male rats. However, in female rats, a significant increase in creatinine was noted at the 500 mg/kg dose ($p = 0.0083$), along with a significant elevation in ALT at 1000 mg/kg ($p = 0.002$) after 28 days of treatment. These sex-specific responses warrant further investigation to better understand potential gender-based variations in toxicity. Throughout the 28-day subchronic study, hematological parameters remained relatively stable in both male and female rats. The extract did not cause significant changes in red or white blood cell counts, hemoglobin levels, or platelet numbers. This suggests that the formulation does not adversely affect the hematopoietic system, which plays a crucial role in physiological balance and serves as an important indicator of systemic health in toxicological assessments [20]. Semi *et al.* (2016) also reported no significant changes in hemoglobin levels, RBCs, WBCs, or platelets between treated and control groups [21]. In female rats, only hematocrit (HCT) was significantly decreased at 500 and 1000 mg/kg ($p < 0.001$ and $p = 0.029$). In male rats, significant decreases were observed in MCH at 500 mg/kg ($p < 0.01$) and 1000 mg/kg ($p < 0.001$), as well as in MCHC at 1000 mg/kg ($p = 0.024$) compared to the control group. These reductions in MCH and MCHC could be linked to various pathophysiological mechanisms. Further studies are needed to determine whether they are related to iron deficiency, oxidative stress, or other underlying causes.

For cytotoxicity assessment, the MTS assay was used to evaluate potential cytotoxic effects of the extract on the NCM cell line and to determine its CC_{50}. According to Malebo *et al.* (2009), a $CC_{50} < 1$ µg/mL indicates high cytotoxicity; 1 - 10 µg/mL, moderate; 10 - 30 µg/mL, low; and >30 µg/mL, non-cytotoxic [22]. The highest tested dose (100 µg/mL) caused only 10.88% cell lethality. In fact, the CC_{50} calculated in our study was 440,457 µg/mL (or 440.46 mg/mL). The studies by Poli *et al.* (2024) corroborate this result. The results from the cytotoxicity tests indicated that the concentration of the hydroethanolic combination extract of *Phyllanthus niruri* (Linn) and *Sida acuta* (Burm) used in treatment against malaria in Togo had a CC_{50} above 100 µg/mL [23]. Similarly, Lee *et al.* (2011) reported no significant cytotoxicity from Phyllanthus species, including aqueous and methanolic extracts of *Phyllanthus amarus*, on normal cells using the MTS assay [24].

5. Conclusion

The results from our acute toxicity study (5000 mg/kg bw), 28-day subchronic toxicity study (500 and 1000 mg/kg bw), and cytotoxicity evaluation of the hydroethanolic extract of the antiviral recipe composed of *Bridelia ferruginea* bark, *Sansevieria liberica* roots, and the whole *Phyllanthus amarus* plant provide plausible scientific evidence regarding its safety. The extract did not cause mortality or major organ toxicity in rats, and it is unlikely to induce hepatic injury or liver dysfunction in acute or 28-day subchronic oral toxicity models.

Acknowledgements

We would like to thank all those who have contributed directly or indirectly to the development of this assessment.

Conflicts of Interest

The authors declare no competing interests.

References

[1] WHO (2017) Global Hepatitis Report 2017. World Health Organization. https://iris.who.int/handle/10665/255016

[2] DSIS and Ministry of Public Health (Togo) (2023) National Report on Viral Hepatitis.

[3] Schweitzer, A., Horn, J., Mikolajczyk, R.T., Krause, G. and Ott, J.J. (2015) Estimations of Worldwide Prevalence of Chronic Hepatitis B Virus Infection: A Systematic Review of Data Published between 1965 and 2013. *The Lancet*, **386**, 1546-1555. https://doi.org/10.1016/s0140-6736(15)61412-x

[4] Mouzou, B.A., *et al*. (2018) Ethnobotanical Survey of Plants Used in the Treatment of Liver Diseases among the Adja People of Togo. *Journal of Scientific Research of University of Lomé*, **20**, 273-284.

[5] Dovi, K., Azoma, K. and Gbeassor, M. (2020) Medicinal Plants Used in Hepatitis Management in Togo: An Ethnobotanical Review. *Phytothérapie*, **18**, 214-220.

[6] Teschke, R. and Eickhoff, A. (2015) Herbal Hepatotoxicity in Traditional and Modern Medicine: Actual Key Issues and New Encouraging Steps. *Frontiers in Pharmacology*, **6**, Article 72. https://doi.org/10.3389/fphar.2015.00072

[7] OECD (2001) Guideline 423 for the Testing of Chemicals: Acute Oral Toxicity—Fixed Dose Procedure.

[8] OECD (2025) Test No. 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing. https://doi.org/10.1787/9789264070684-en

[9] Lau, C.B.S., Ho, C.Y., Kim, C.F., Leung, K.N., Fung, K.P., Tse, T.F., *et al*. (2004) Cytotoxic Activities of Coriolus Versicolor (Yunzhi) Extract on Human Leukemia and Lymphoma Cells by Induction of Apoptosis. *Life Sciences*, **75**, 797-808. https://doi.org/10.1016/j.lfs.2004.04.001

[10] Hoinsou, Y., Poli, S., Koula, F.C., Gbati, L., *et al*. (2024) Toxicological Study of the Hydroalcoholic Extract of a Recipe of Three Plant Used in Traditional Togolese Medicine. *Journal of Biosciences and Medicines*, **12**, 270-280.

[11] Ingenbleek, L., Lautz, L.S., Dervilly, G., Darney, K., *et al*. (2025) Risk Assessment of

Chemicals in Food and Feed: Principles, Applications and Future Perspectives. https://books.rsc.org/books/edited-volume/891/chapter/676121/Risk-Assessment-of-Chemicals-in-Food-and-Feed

[12] Bakoma, B., Berké, B., Eklu-Gadegbeku, K., Agbonon, A., Aklikokou, K., Gbeassor, M., *et al*. (2014) Effect of *Bridelia ferruginea* Benth (Euphorbiaceae) Ethyl Acetate and Acetone Fractions on Insulin Resistance in Fructose Drinking Mice. *Journal of Ethnopharmacology*, **153**, 896-899. https://doi.org/10.1016/j.jep.2014.03.065

[13] Lawson-Evi, P., Eklu-Gadegbeku, K., Agbonon, A., *et al*. (2008) Toxicological Assessment on EXtracts of *Phyllanthus amarus* Schum and Thonn. *Scientific Research and Essays*, **3**, 410-415.

[14] Tchakondo, T. (2003) Circadian and Circannual Rhythms in Sodium Nitroprusside Toxicity in Mice: A Chronobiometric and Modeling Approach. Doctoral Thesis, University of Carthage.

[15] Akinyele, B.T. and Dada, E.O. Effects of Ethanol Extract of *Bridelia ferruginea* (W.) on the Haematological and Histopathological Parameters in Swiss Albino Rats Infected with Salmonella Typhi.

[16] Cattley, R.C. and Cullen, J.M. (2013) Liver and Gall Bladder. In: *Haschek and Rousseaux's Handbook of Toxicologic Pathology*, Elsevier, 1509-1066. https://doi.org/10.1016/B978-0-12-415759-0.00045-5

[17] Oduola, T., Muhammad, A., Aiyelabegan, F., Tajudeen, M. and Okalawon, S. (2018) Hepatotoxic Assessment of *Phyllanthus amarus* Leaf Extract in Wistar Rats. *European Journal of Medicinal Plants*, **23**, 1-11. https://doi.org/10.9734/EJMP/2018/41238

[18] Bakoma, B., Berké, B., Eklu-Gadegbeku, K., *et al*. (2013) Acute and Subchronic (28-Day) Oral Toxicity of *Bridelia ferruginea* Root Bark Extract in Rodents. *Food and Chemical Toxicology*, **52**, 176-179. https://doi.org/10.1016/j.fct.2012.11.021

[19] Amida, M.B., Yemitan, O.K. and Adeyemi, O.O. (2007) Toxicological Evaluation of Aqueous Root Extract of *Sansevieria liberica*. *Journal of Ethnopharmacology*, **113**, 171-175.

[20] Nene, B.S.A., *et al*. (2009) Phytochemical and Pharmacological Study of *Bridelia ferruginea* on Guinea Pig Intestinal Motility. *Scientific African*, **5**, 305-320.

[21] Nene-Bi, S.A., Ramachandran, V., Vengal, R.P., Gopalakrishnan, R., Dhanabal, S.P. and Traore, F. (2016) Subchronic Toxicity Studies of the Aqueous Stem Bark Extract of *Bridelia ferruginea* in Wistar Rats. *Bulletin of Environment, Pharmacology and Life Sciences*, **5**, 14-21.

[22] Malebo, H.M., Tanja, W., Cal, M., Swaleh, S.A.M., Omolo, M.O., Hassanali, A., *et al*. (2009) Antiplasmodial, Anti-Trypanosomal, Anti-Leishmanial and Cytotoxicity Activity of Selected Tanzanian Medicinal Plants. *Tanzania Journal of Health Research*, **11**, 226-234. https://doi.org/10.4314/thrb.v11i4.50194

[23] Poli, S., Ataba, E., Gbekley, E.F., Alognon, A., *et al*. (2024) Antiplasmodial, Antioxidant, Antipyretic and Cyto-Toxic Activities of Hydroethanolic Extract of *Phyllanthus niruri* (Linn), *Sida acuta* (Burm) and Their Combination. *International Journal of Biological and Chemical Sciences*, **18**, 1199-1211. https://doi.org/10.4314/ijbcs.v18i4.1

[24] Lee, S.H., Jaganath, I.B., Wang, S.M. and Sekaran, S.D. (2011) Antimetastatic Effects of Phyllanthus on Human Lung (A549) and Breast (MCF-7) Cancer Cell Lines. *PLOS ONE*, **6**, e20994. https://doi.org/10.1371/journal.pone.0020994