

Research Article

Cytotoxicity, Acute and Subacute Toxicity Evaluation of Hydroethanolic Extract of *Dialium Guineense* Willd Fruit Pulp

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Abstract

Objective: This study aims to evaluate the acute and subacute toxicity, as well as the cytotoxicity of the hydroethanolic extract of *D. guineense* willd fruit pulp. **Methodology:** Three different tests were performed. The cytotoxicity test was performed using *Artemia salina* larvae. The acute toxicity test was conducted by single oral administration of *Dialium guineense* at 5000 mg/kg. When subacute toxicity tests were performed by repeated oral administration of *Dialium guineense* at 250, 500, and 1000 mg/kg/day for 28 days. **Results:** The fruit pulp of *Dialium guineense* presented a CL₅₀ equal to 1.40 mg/mL and an LD₅₀ greater than 5000 mg/kg. This pulp at the dose of 1000 mg/kg significantly reduced blood glucose as well as the overall white blood cell counts, particularly neutrophils and eosinophil polymorphonuclear cells, at low doses after 28 days of treatment. **Conclusion:** The hydroethanolic extract of the *Dialium guineense* willd fruit pulp does not show signs of cytotoxicity, major acute or subacute toxicity.

Keywords

Dialium Guineense Willd Fruit, Cytotoxicity, Acute Toxicity, Subacute Toxicity

1. Introduction

Dialium guineense (*D. guineense*) known as black tamarind is a legume in the *Fabaceae* family [1, 2]. The pulp of its fruit is highly prized by all socio-economic strata in Togo. Unfortunately, a good portion of consumers report constipation following their intake. Our previous work has confirmed

this claim [3]. Other of our previous work has also shown that the pulp of the fruit of *D. guineense* has antidiarrheal activities in Wistar rats [4]. The work of [5-7] have shown that *D. guineense* is a medicinal plant with great potential. According to the same source, it is a valuable and untapped

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resource of active drugs to combat various diseases. But unfortunately, there is little scientific data to rule on the toxicity of *D. guineense* fruit pulp. This study therefore aims to evaluate the acute and subacute toxicity of 28 days on Wistar rats, as well as the larval toxicity on *Artemia salina* larvae of the hydroethanolic extract of *D. guineense* fruit pulp, in order to have an insight on their toxicity before their uses.

2. Materials and Methods

2.1. Collection and Extraction of Plant Material

For our study, the fruit pulp of *D. guineense* was used. The fruits of *D. guineense* were purchased at the market in Mission-Tov é a village located about twenty kilometres north of the city of Lomé. A control specimen was preserved in the herbarium of the laboratory of botany and plant ecology of the Faculty of Sciences (FDS) of the University of Lomé under the reference Togo - 15879.

2.2. Extraction

To obtain the pulp, the fruits covered with a hard, dark red shell were broken open to expose the soft, orange pulp. This pulp was carefully peeled and then dried at room temperature to remove its water content. Finally, the dried pulp was ground into powder. Subsequently, this powder was macerated in a mixture of ethanol and water (80/20) for 72 hours under automatic stirring on an orbital shaker (IKA KS 501 Orbital Digital Platform Shaker). After double filtration using cotton and Whatman paper, the obtained filtrate was evaporated at 45 °C using a rotary evaporator (IKA RV 10 Digital).

2.3. Treatment of Animals

Wistar rats weighing 150-170 g (3-4 months old) and *Artemia salina* larvae were used.

Rats were obtained from the animal facility of the Department of Pharmacology and Toxicology, Faculty of Health Sciences (FSS), University of Lomé Togo. These animals were treated in accordance with the Organization for Economic Cooperation and Development (OECD) standards [8]. They were kept at a temperature of 23 ± 3 °C during a 12-h light/dark cycle, with free access to water and food (except on experimental days). Animals were acclimatised to laboratory conditions for one week before each experiment. Studies were performed in the toxicology laboratory of the FSS, University of Lomé Togo.

2.4. Cytotoxicity

Meyer's method [9] modified by Dossou-Yovo et al, [10] was used. For this test, 500 milligrams of *Artemia salina* larvae was incubated in 500 mL of seawater for 48 hours under mechanical agitation. The larvae were distributed among 10 tubes of different concentrations (from 25 to 0.05

mg/mL). Each tube contained 16 larvae in 2 mL of *D. guineense* solution at decreasing concentrations. An 11th tube containing 2 mL of seawater and also 16 larvae served as a control. The tubes were placed under automatic agitation for 24 hours, then observed to determine the mortality of the larvae. This mortality was defined as their immobility for 30 seconds.

2.5. Acute Toxicity

Acute toxicity was conducted according to OECD's 423 Tests Guidelines [8]. A test limits dose of 5000 mg/kg was achieved with three female Wistar rats. The extract was orally administered to each animal sequentially within 48 hours. These animals were observed individually thirty minutes after administration, for the first 24 hours, for signs of acute toxicity or changes in behaviour. They were then observed at least once a day for 14 days.

2.6. Subacute Toxicity

The subacute toxicity study was carried out according to [11] with a few modifications. To do this, the animals were divided into four groups of four rats, with two males and two females per group. The first group (group 1) received distilled water and served as the control group. The 2nd, 3rd, and 4th groups (groups 2, 3, and 4) received the extract of *D. guineense* fruit pulp at doses of 250; 500; and 1000 mg/kg body weight of extract, respectively. The extract was administered daily for 28 days simultaneously. The rats after administration were observed at least twice a day for morbidity and mortality. Their body weight was recorded daily. Mobility, aggression, food intake as well as the appearance and number of feces were observed in each group. On day 29, all rats were fasted for 12 hours and then anaesthetised with ether. After anaesthesia, blood samples were collected from the retroorbital sinus into dry tubes for biochemical analyses and into ethylenediaminetetraacetic acid (EDTA) tubes for haematological analyses.

2.6.1. Evaluation of Haematological and Biochemical Parameters

The myndre automaton was used to assess biochemical parameters such as alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and blood glucose. For haematological parameters, white blood cell count (WBC), red blood cell count (RBC), haematocrit (HCT), haemoglobin (HB), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), platelet count (PLT), and mean corpuscular volume (MCV) were performed.

2.6.2. Relative Weight of Organs

After blood collection, each rat was euthanized with ether and then dissected for a macroscopic examination. Organs such as the liver, kidneys, heart, and spleen were isolated and weighed. Their relative weight was calculated using the fol-

lowing equation:

$$\text{Relative weight} = \frac{\text{Weight of the organ}}{\text{body weight on the day of sacrifice}} \times 100.$$

The samples were subsequently transferred into 10% formalin solutions for histopathological studies.

2.6.3. Histopathological Studies

The Laboratory of the Department of Anatomy-Pathology of the Faculty of Health Sciences (FSS) of the University of Kara served as a study setting for this study. The liver and kidneys underwent standard histology techniques after a macroscopic examination. The HIS-stained slides were subsequently interpreted by a pathologist with a LEICA DM 1000 microscope at different magnifications; then the images were taken using a LEICA ICC₅₀ camera.

2.7. Study of Phytochemical Composition

The study of the phytochemical composition was carried out with the aim of researching the main chemical groups such as alkaloids, tannins, sterols, triterpenes, phenols, flavonoids, saponins, hydrocarbons, carbohydrates, etc. This research was carried out using appropriate methods in the pharmacognosy laboratory of the Faculty of Health Sciences of the University of Lomé

2.8. Statistical Analysis

Results are expressed as mean $\pm$ standard error of the mean (SEM) for each group. Data were analysed using SPSS version 26 software. All pooled data were statistically evaluated and the significance of various treatments was calculated using one-way analysis of variances ANOVA followed by the post hoc honesty difference test Turkey's significant difference (HSD). A *P* value

<0.05 was considered statistically significant.

3. Results

3.1. Preparation of Plant Material

Regarding the plant material, 600.17 g of *D. guineense* fruit pulp powder was obtained. After extraction and evaporation, 138.42 g of extract was obtained, giving a yield of 23.06%.

3.2. Cytotoxicity

With the number of dead larvae in each tube, we were able to plot the logarithmic trend curve expressing the number of dead larvae as a function of the *D. guineense* concentration (Figure 1). From this trend curve, the determination of the lethal concentration of 50 (LC₅₀) was carried out. This LC₅₀ is 1.40 mg/mL of *D. guineense* fruit pulp.

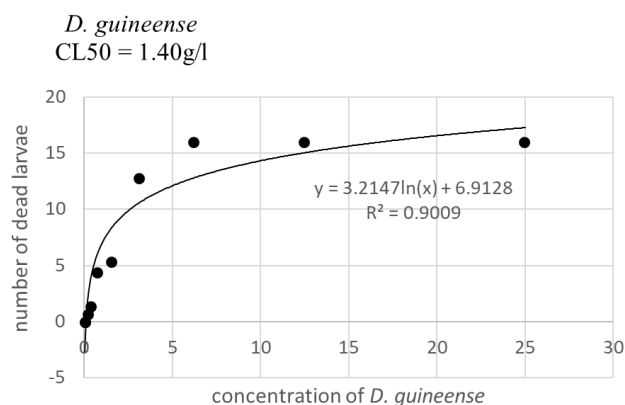


Figure 1. Effects of *D. guineense* fruit pulp on *Artemia salina* larval mortality.

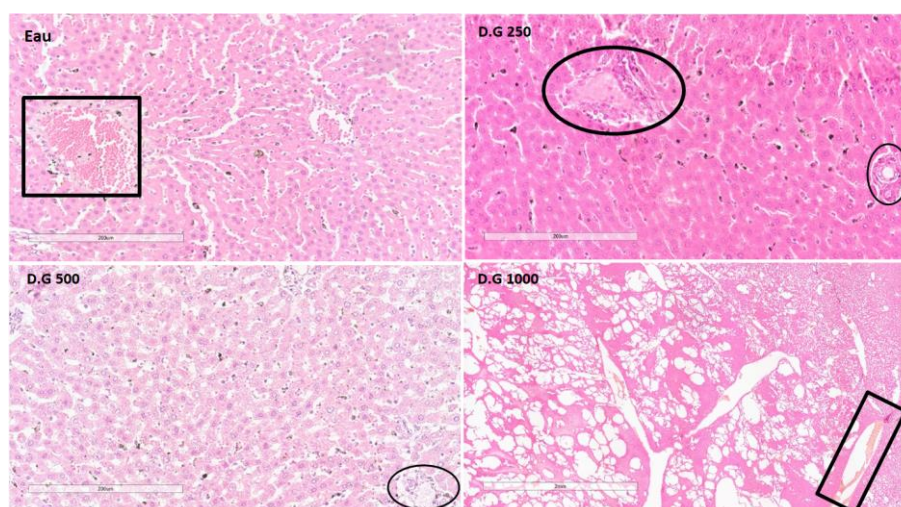


Figure 2. Effect of *D. guineense* (D.G) pulp extract on the histological structure of the liver in rats after 28 days of treatment: ○: oedema; □: congestion.

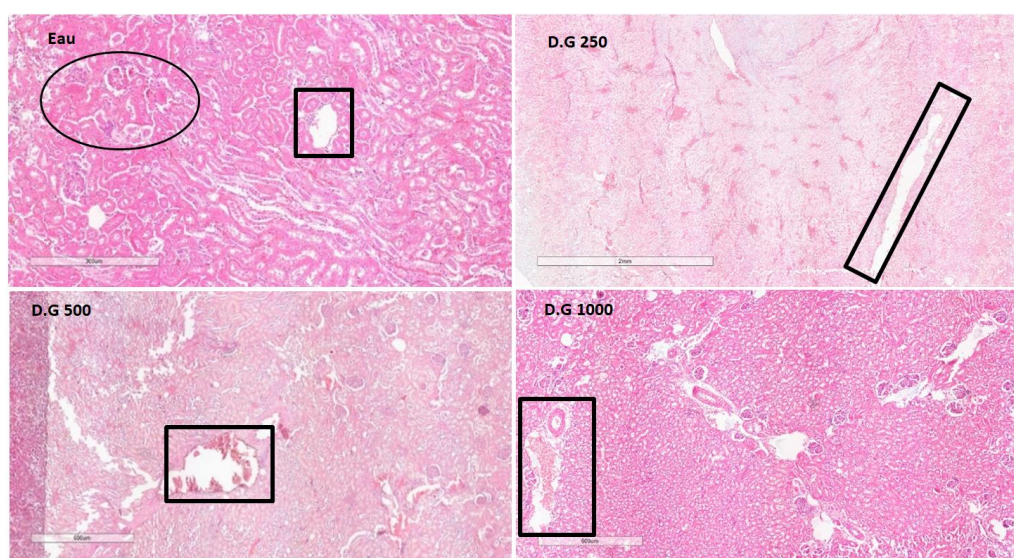


Figure 3. Effect of *D. guineense* (D.G) pulp extract on the histological structure of kidneys in rats after 28 days of treatment: ○: oedema; □: congestion.

3.3. Acute Toxicity

Whether over a short (48 hours) or prolonged (14 days) observation period, administration of the limit dose of 5000 mg/kg did not result in any signs of acute toxicity or mortality in the three treated rats. Therefore, the LD₅₀ of the hydroethanolic extract of *D. guineense* fruit pulp is greater than 5000 mg/kg.

3.4. Subacute Toxicity

3.4.1. Clinical Observation

Oral administration of the extract for 28 days did not induce any behavioural changes in the treated rats. No cases of mortality or adverse effects were observed throughout the experimental period.

As shown in Table 1, there was no significant variation in the mean body weight of treated and control rats during the 28 days of treatment.

3.4.2. Relative Weight of Organs

Table 2 shows the variation in the relative weight of some organs in rats after 28 days of treatment. There was a slight increase in the relative weight of the liver, kidneys and spleen at the dose of 1000 mg/kg. On the other hand, the relative weight of the heart remained stable.

3.4.3. Biochemical Parameters

Table 3 shows the effect of *D. guineense* pulp extract on blood glucose and liver enzymes (ALAT, ASAT) in Wistar rats after 28 days of treatment. A significant decrease ($p < 0.05$) in blood glucose levels can be noted at the dose of 1000 mg/kg. On the other hand, the variations in ALAT and ASAT

levels between the treated and untreated groups remain non-significant.

3.4.4. Haematological Parameters

(i). Leukocyte Parameters

Table 4 shows the effect of *D. guineense* pulp extract on leukocyte parameters in rats, after 28 days of treatment. It is noted a decrease in the level of white blood cells (WBC), polymorphonuclear neutrophils (PNN) and monocytes (MON) in the treated group with low doses. On the other hand, the levels of eosinophilic polymorphonuclear leukocytes (PNE) and basophils (PNB), as well as that of lymphocytes (LYMP) do not show significant differences between the groups.

(ii). Erythrocyte Parameters

Table 5 presents the effect of *D. guineense* pulp extract on erythrocyte parameters (HGB, HCT, MCV, MCH, MCHC) in rats Wistar after 28 days of treatment. No significant differences were observed between the treated groups and the control group for all of these parameters.

3.4.5. Histopathological Studies

Histologic sections show the presence of some slight oedema and congestion in the kidneys and livers in the treated and untreated groups.

3.5. Phytochemical Composition

Phytochemical screening (Table 6) of hydroethanolic extracts revealed the presence of flavonoids, phenols, carbohydrates, sterols, triterpenes, and tannins. However, the absence of alkaloids, saponins, and quinones was noted.

Table 1. Change in body weight of rats during the 28 days of treatment.

	Distilled water negative control	<i>D. guineense</i>		
		250 mg/kg	500 mg/kg	1000 mg/kg
Day 1	155.75 ± 14.23	157.00 ± 14.15	158.75 ± 11.20	163.00 ± 4.44
Day 3	158.25 ± 14.70	158.00 ± 12.77	162.00 ± 9.96	166.50 ± 2.72
Day 6	159.00 ± 14.92	162.75 ± 15.88	162.25 ± 10.12	170.50 ± 2.72
Day 9	167.50 ± 14.95	168.25 ± 16.13	163.25 ± 9.28	173.50 ± 5.32
Day 12	165.00 ± 15.17	169.75 ± 15.09	177.25 ± 10.27	180.00 ± 8.80
Day 15	166.25 ± 15.65	182.00 ± 19.13	178.75 ± 7.18	176.75 ± 7.21
Day 18	173.00 ± 16.72	185.25 ± 20.01	187.25 ± 8.75	181.50 ± 6.22
Day 21	174.25 ± 16.24	187.50 ± 19.87	185.25 ± 7.38	180.50 ± 7.96
Day 25	178.75 ± 16.22	193.00 ± 21.70	177.50 ± 7.96	182.00 ± 8.01
Day 28	181.25 ± 14.26	192.75 ± 23.35	175.25 ± 7.18	181.50 ± 8.25

Results are expressed as mean ± SEM (Standard Error of the Mean) with n = 4; degree of significance * P < 0.05; ** P < 0.01; *** P < 0.001 versus the negative control group (one-way ANOVA followed by Turkey's multiple comparison test).

Table 2. Relative organ weight after 28 days of treatment.

	Distilled water negative controls	<i>D. guineense</i>		
		250 mg/kg	500 mg/kg	1000 mg/kg
Liver	3.00 ± 0.17	2.68 ± 0.16	2.91 ± 0.16	3.44 ± 0.17
Kidney	0.53 ± 0.04	0.52 ± 0.02	0.56 ± 0.04	0.61 ± 0.04
Heart	0.35 ± 0.02	0.34 ± 0.02	0.37 ± 0.02	0.38 ± 0.02
Rate	0.26 ± 0.03	0.28 ± 0.03	0.30 ± 0.06	0.32 ± 0.09

Results are expressed as mean ± SEM (Standard Error of the Mean) with n = 4; degree of significance * P < 0.05; ** P < 0.01; *** P < 0.001 versus the negative control group (one-way ANOVA followed by Turkey's multiple comparison test).

Table 3. Some biochemical parameters of rats after 28 days of treatment.

	Distilled water (negative control)	<i>D. guineense</i>		
		250 mg/kg	500 mg/kg	1000 mg/kg
ALAT (IU/L)	63.00 ± 10.65	76.00 ± 6.79	76 ± 15.04	55.25 ± 6.85
ASAT (UI/L)	185.50 ± 18.85	179.25 ± 4.48	156.25 ± 28.89	157.25 ± 4.40
Blood sugar (g/L)	1.04 ± 0.07	0.79 ± 0.08	0.79 ± 0.05	0.67 ± 0.11 *

Results are expressed as mean ± SEM (Standard Error of the Mean) with n = 4; Degree of significance * P < 0.05; ** P < 0.01; *** P < 0.001 versus negative control group (one-way ANOVA followed by Tukey's multiple comparison test).

Table 4. Leukocyte formula of rats after 28 days of treatment.

	Distilled water negative control	<i>D. guineense</i>		
		250 mg/kg	500 mg/kg	1000 mg/kg
WBC (/mm ³)	21.72 ± 0.78	19.13 ± 1.71	15.20 ± 2.35 *	19.58 ± 0.72
LYMP (/mm ³)	61.50 ± 1.04	62.50 ± 4.17	63.00 ± 3.89	57.25 ± 6.92
GNP (/mm ³)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
PNE (/mm ³)	3.75 ± 0.75	1.50 ± 0.55 *	2.75 ± 0.48	3.50 ± 0.65
PNN (/mm ³)	29.00 ± 1.29	31.50 ± 4.63	29.50 ± 4.17	35.25 ± 6.12
MON (/mm ³)	3.75 ± 0.75	1.50 ± 0.646 *	2.75 ± 0.48	3.50 ± 0.65

Results are expressed as mean ± SEM (Standard Error of the Mean) with n = 4; degree of significance * P < 0.05; ** P < 0.01; *** P < 0.001 versus the negative control group (one-way ANOVA followed by Turkey's multiple comparison test).

WBC: Leukocytes, PNN: Neutrophilic polymorphonuclear cells, PNE: Eosinophilic polymorphonuclear cells, PNB: Basophilic polymorphonuclear cells, LYMP: Lymphocytes, MON: monocytes

Table 5. Erythrocyte formula.

	Distilled water negative control	<i>D. guineense</i>		
		250 mg/kg	500 mg/kg	1000 mg/kg
RBC / mm ³	9.18 ± 0.15	9.18 ± 0.14	8.54 ± 0.69	8.81 ± 0.17
HGB (g/dl)	14.63 ± 0.17	15.83 ± 0.23	14.35 ± 0.72	14.73 ± 0.44
HCT (%)	47.97 ± 0.54	51.73 ± 0.82	46.50 ± 1.94	48.62 ± 1.51
MCV (fl)	52.20 ± 0.25	54.05 ± 1.78	55.05 ± 2.60	55.20 ± 0.64
MCH (Pg)	15.95 ± 0.12	16.52 ± 0.45	16.92 ± 0.61	16.72 ± 0.19
MCHC (g/dl)	30.47 ± 0.17	30.60 ± 0.27	30.80 ± 0.51	30.27 ± 0.14

Results are expressed as mean ± SEM (Standard Error of the Mean) with n = 4; degree of significance * P < 0.05; ** P < 0.01; *** P < 0.001 versus the negative control group (one-way ANOVA followed by Turkey's multiple comparison test).

Table 6. Screening phytochemicals of hydroethanolic extracts of *D. guineense* fruit pulp.

Families of compounds	Presence in the fruit pulp of <i>D. guineense</i>
Reducing sweets	+
Phenols	+
Flavonoids	+
Cardiotonic glycosides	+
Condensed tannins	+
Hydrolyzable tannins	+
Sterols	+
Triterpenes	+
Alkaloids	-

Families of compounds	Presence in the fruit pulp of <i>D. guineense</i>
Quinones	-
Saponins	-

+: present, -: absent

4. Discussions

This study aimed to evaluate the acute and subacute 28-day toxicity of the hydroethanolic extract of *D. guineense* fruit pulp on the Wistar rat, as well as its cytotoxicity on the *Artemia salina* larva, in order to gain insight into its safety before use.

For this study, no animals showed signs of mortality or morbidity after administration of the single dose of 5000 mg/kg. Thus, according to [8], the median lethal dose (LD₅₀) of *D. guineense* fruit pulp is greater than 5000 mg/kg.

Regarding subacute toxicity, we did not observe any case of mortality or morbidity during the 28 days of treatment. An increase in the body weight of rats was noted in both the treated and control groups. This increase would be due to the growth of the rats. Because the animals used are young, growing animals. On the other hand, we recorded a slight increase in the relative weight of the liver, kidneys and spleen at the dose of 1000 mg/kg. But the relative weight of the heart remained stable. This confirms an absence of cardiotoxic effect extract of *D. guineense* [12, 13]. Despite this slight increase in liver weight, the liver enzymes ALAT and ASAT are not elevated. This slight hepatic and renal hypertrophy may be an adaptive response to the presence of metabolically active compounds such as flavonoids, phenols, triterpenes ... [14, 15].

Although ALAT and ASAT did not vary, our results revealed a significant decrease in blood sugar at the dose of 1000 mg/kg. The phytochemical study revealed the presence of groups such as flavonoids, phenols, carbohydrates, sterols, triterpenes, etc. According to the work of [16-19], flavonoids decrease considerably the activity of hepatic glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. They increase plasma levels of insulin, C-peptide and leptin, thus decreasing blood glucose. The triterpenes according to the work of [20, 21] induce low expression of p-GSK-3 β (Phosphorylated Glycogen Synthase Kinase-3 beta), p-AKT (Serine/Threonine Kinase) and GLUT-4 (Glucose Transporter Type 4), as well as higher expression of IRS-1 ser307. In addition, triterpenes reduce postprandial blood glucose via inhibition of α -glucosidase, SGLT-1 (Sodium-Glucose Linked Transporter 1), GLUT-2 (Glucose Transporter Type 2) and amylase [22, 23]. This may explain the hypoglycaemic activity of *D. guineense* fruit pulp despite its high carbohydrate and lipid content [7, 24-26].

Our results revealed a slight dose-dependent increase in relative spleen weight in the treated groups. This increase may

be due to a mild immunomodulatory effect, with no signs of toxicity. This immunomodulatory effect would itself be due to the effects of flavonoids, tannins and other chemical groups [27-29]. This plant immunomodulatory effect can be materialised by a significant decrease in the level of white blood cells (WBC), eosinophilic polymorphonuclear leukocytes (EPL), and monocytes (MON) in the groups treated with *D. guineense* pulp at low doses. This shows that *D. guineense* pulp at low doses has anti-inflammatory and/or immunosuppressive properties [27, 28, 30-32], but shows signs of toxicity at high doses.

The non-toxicity of *D. guineense* pulp is confirmed by the absence of lesions or deformations of the liver or kidneys observed on the histological sections. The latter only shows some congestion and oedema. These slight oedema and congestion will not be linked to the *D. guineense* extracts, because they are present in the treated groups and the controls.

The LC₅₀ (Lethal Concentration 50) is the concentration of a substance that causes the death of 50% of the individuals exposed under given experimental conditions [33]. It is an index of acute toxicity [33]. A low LC₅₀ indicates high toxicity, while a high LC₅₀ indicates moderate or low toxicity [33, 34]. For our study, the LC₅₀ of *D. guineense* fruit pulp on *Artemia salina* larvae is equal to 1.40 mg/mL. This value is greater than 0.1 mg/mL, which is the maximum value beyond which an extract can be considered non-cytotoxic [10, 35]. The LC₅₀ of *D. guineense* fruit pulp is significantly higher and therefore less toxic than those reported by Nerdy et al, (2021) on *Piper betle* (45 μ g/mL) [36] or *Dillenia serrata* (9-58 μ g/mL) [37]. It is however comparable to the extracts deemed to be of low toxicity, such as *Bintaro* (*Cerbera* sp.) with 1.199mg/mL [38] or hydroalcoholic extract from the root of *Carissa spinarum* which is 0.9mg/mL [10]. This confirms the non-toxicity of *D. guineense* fruit pulp.

5. Conclusion

The fruit pulp of *D. guineense* did not show signs of cytotoxicity on *Artemia salina* larvae, nor major acute or subacute toxicity in Wistar rats. This pulp after 28 days of treatment nevertheless induced hypoglycaemia at high doses, and immunosuppressive effects with low doses. Although further studies are necessary to use different models, we can say that the fruit pulp of *D. guineense* can be used for nutritional and therapeutic purposes. In addition, this pulp should not be consumed by people suffering from chronic hypoglycaemia or

in a situation of immunosuppression for a long period.

Abbreviations

ALAT	Alanine Aminotransferase
ASAT	Aspartate Aminotransferase
<i>D. guineense</i>	<i>Dialium guineense</i>
EDTA	Ethylenediaminetetraacetic Acid
GLUT-2	Glucose Transporter Type-2
GLUT-4	Glucose Transporter Type-4
HB	Haemoglobin
LYMP	Lymphocytes
LC ₅₀	Median Lethal Concentration
LD ₅₀	Median Lethal Dose
MCV	Mean Corpuscular Volume
MCHC	Mean Corpuscular Haemoglobin Concentration
MON	Monocytes
OECD	Organization for Economic Cooperation and Development
p-AKT	Serine/Threonine Kinase
PLT	Platelet
p-GSK-3β	Phosphorylated Glycogen Synthase Kinase-3 beta
PNB	Basophils Polymorphonuclear
PNE	Eosinophilic Polymorphonuclear
PNN	Neutrophils Polymorphonuclear
SEM	Standard Error of the Mean
WBC	White Blood Cells

Ethical Approval

The study was conducted following an approved animal use protocol from the Institutional Ethical Committee for Teaching and Research (ref no. CNCB- CEER 2801/2010).

Conflicts of Interest

The authors declare no conflicts of interest.

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