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Phytochemical study and toxicological assessment of hydroethanolic extract of *Dichapetalum guineense* (DC.) keay leaves

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Abstract
Plant extracts contain a variety of chemical compounds with different biological activities. Some of these extracts may be toxic to human health. The aim of this study was to carry out phytochemical screening and assess the toxicity of the hydroethanolic extract of the leaves of *Dichapetalum guineense* (DC.) Keay. The phytochemical study has been carried out using qualitative methods. In the acute toxicity study, a single dose of 5000 mg/kg of *D. guineense* was orally administered to healthy Wistar rats. Rats were observed for mortality and clinical signs for few hours, then daily for 14 days. In the subchronic toxicity study, 300 and 600 mg/kg were orally administered to rats daily for 28 consecutive days. At the end of the treatment, rats were sacrificed for haematological, biochemical and histological examinations. Results showed that the hydroethanol extract contained terpenoids, coumarins, mucilages, anthraquinones, tannins and flavonoids, with a notable absence of alkaloids. The extract was non-toxic at a single dose of 5000 mg/kg. No abnormalities were observed in any of the rats treated. The LD_{50} is greater than 5000 mg/kg. After daily administration of repeated doses of 300 and 600 mg/kg, the extract did not cause any significant change in the relative organ weights. The majority of biochemical and haematological parameters were not affected by the extract. However, the extract induced a significant increase in glycaemia at 600 mg/kg. Histological observations did not show any significant abnormality. Overall, results show that *D. gueneens* is relatively safe.

Keywords: *Dichapetalum guineennse*, hydroethanolic extract, phytochemistry, toxicity, wistar rats

1. Introduction
Medicinal plants and their various extracts contain secondary metabolites responsible for their therapeutic properties in the treatment of disease.
Although herbal healthcare is considered safe, some are known to be toxic at high doses and others may have a potential negative effect after prolonged use. Indeed, several studies carried out on traditional herbal treatments reported problems of toxicity or interaction that can cause therapeutic failures [1, 2].
Ephedra sinica is a plant used in traditional Chinese medicine for its cardiovascular properties. During its use for weight loss, it has led to numerous cases of cardiovascular accidents, cerebrovascular accidents and psychiatric disorders. This is why its use has been banned in Europe [3, 4]. There have also been reports of poisoning by *Callilepis laureola*, a plant used in the traditional treatment of digestive disorders and infertility, which is widespread in South Africa. This plant has caused many deaths among the population. It has been incriminated in the occurrence of cardiovascular disorders and hepatitis with major hepatocellular necrosis during autopsies of more than 1,500 people who died in the province of Kwazulu-Natal [4, 5].
Dichapetalum guineense (Caesalpiniaceae, synonym, *Dichapetalum madagascariense*) is a tropical plant widespread in West Africa (Ghana, Togo, Benin, etc.). It is widely used in traditional medicine to treat a number of illnesses, including malaria [6] and high blood pressure [7]. It is also used to treat diabetes in pregnant women in Benin and hypertension [8]. In addition, à molecule called Dichapetain A has been isolated from the root of the plant in Ghana [9]. Despite its widespread use, little toxicological data are available on the safety of repeated exposure to *D. guineense* leaves. As part of a safety assessment of the hydroethanol extract of *D. guineense* leaves, phytochemical and toxicological studies were therefore carried out to investigate its potential toxicity after single and repeated oral administration for 28 days in wistar rats.

2. Materials and Methods

2.1. Plant material

The plant (*Dichapetalum guineense*) was collected in March 2023 in Lomé (Togo). Botanical authentication was confirmed at the Department of Botany, University of Lomé, where a coupon specimen of *D. guineense* was deposited in the herbarium of the Togolese flora (TOGO15929).

2.2. Phytochemical screening

The main chemical groups in the plant (leaf powder) were screened using solubility tests, colouring and precipitation reactions and ultraviolet light tests [10, 11].

2.3 Preparation of crude extracts

D. guineense extract powder (100 g) was poured at room temperature into one litre of ethanol-water mixture (70/30 v/v) for 72 h, with regular stirring. The resulting mixture was filtered and the filtrate concentrated by evaporating the ethanol-water mixture at 40°C under reduced pressure using a rotary evaporator (BUCHI, Switzerland).

2.4 Animals

Healthy Wistar rats of both sexes weighing between 90 and 150 g obtained from the animal physiology laboratory of the University of Lomé were used for acute and sub-chronic toxicity studies. Animals were housed in a room and maintained under standard environmental conditions of the 12/12 h light/dark cycle. They were housed in cages and fed regularly. The experiment was performed in accordance with the guidelines established by the OECD [12]. Animal use and handling received the approval of the institutional ethics committee of the University of Lomé (N° SBM/UL/15/NS0009).

2.5 Acute oral toxicity

Acute toxicity study of *D. guineense* leaf extract was conducted in accordance with guideline 423 of the Organisation for Economic Co-operation and Development (OECD) [12]. A total of eight (8) female rats divided into two groups of four (4) were used. The first (control group) received distilled water, while the second group received à single oral dose of 5000 mg/kg body weight extract. The animals were kept fasting for 12 hours before administration of the extract by gavage, and the rats were held for a further 3 to 4 hours. The animals were observed individually at least once every 30 minutes after administration, periodically during the first 24 h (with particular attention during the first 4 hours) and daily for a period of 14 days. Behavioural observations included mobility, agitation, respiration, asthenia, changes (hair, eyes and mucous membranes), tremor, convulsion, faecal appearance, lethargy, sleep and coma. Animals that died during the observation period were subjected to autopsy. On day 15, all surviving animals were sacrificed and vital organs such as the lungs, heart, liver and kidneys were removed and subjected to macroscopic observation.

2.6 Subchronic oral toxicity

Subchronic oral toxicity of the hydroethanol extract was assessed using the OECD protocol [13]. Fifteen (15) Wistar rats were divided into three (03) groups of 5 individuals (3 males and 2 females). Two (2) groups received respectively the doses of 300 and 600 mg/kg body weight (bw) of the hydroethanol extract of *D. guineense* daily for 28 consecutive days. The 3rd group (control group) was treated with distilled water over the same period. The rats' behaviour was observed every day and their weight measured every two (02) days. Blood was collected from each animal on day 29 by retro-orbital puncture. For biochemical tests, blood was collected in dry tubes for haematological tests, blood was collected in tubes containing EDTA. All animals were then sacrificed and organs such as the heart, lungs, liver, spleen and kidneys were removed, weighed and macroscopically examined for lesions or signs of toxicity. Relative organ weights were determined using the following formula.

RW = (Organ weight/Body weight per rat) x 100.

2.7 Determination of haematological parameters

Blood samples collected in tubes containing EDTA were used to determine the contents s of white blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), platelets (PLT) and haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin content (MCHC) and mean corpuscular haemoglobin concentration (MCHC). These haematological parameters were determined using an automatic haematology analysé (Mindray BC-2800, China).

2.8 Determination of biochemical parameters

Blood samples collected in tubes without anticoagulant were centrifuged at 3000 rpm for 10 min. The collected sera, stored at -20 °C, were used to assay enzymes such as transaminases (alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT)), alkaline phosphatase (ALP), total protein (TP), total bilirubin (BT), total cholesterol and triglycerides as biochemical which are indicators of liver damage and/or dysfunction. Kidney dysfunction was assessed in by measuring creatinine and blood urea levels. Glucose levels were measured to assess pancreatic function. Standard diagnostic kits purchased from Human GmbH. D-65205 (Wiesbaden, Germany) were used for spectrophotometric determination of biochemical parameters.

2.9 Histological sections

After the rats were sacrificed, the organs (lungs, heart, liver, kidneys) were removed, washed with fresh 0.09% saline solution and preserved in 10% formalin. The organs were examined macroscopically (Leica DM1000, Germany) to note any changes in colour or shape. Histological sections stained haematoxylin-eosin.were observed microscopically to detect any microscopical change in organs architecture.

2.10 Statistical analysis

All statistical analyses were performed using GraphPad ® Prism 8.4.3 software. Results are expressed as mean±standard error of the mean (SEM). The statistical study was carried out by one-way analysis of variance (ANOVA) followed by Tukey's test to assess significant differences between groups. Values are considered significantly different when $p<0.05$.

3. Results

3.1 Phytochemical screening

The phytochemical study carried out showed that both types of extract (hydroethanolic and aqueous) of *D. guineense* contain secondary metabolites such as flavonoids, anthraquinones, terpenoids, tannins, reducing compounds, mucilages and coumarins (Table 1). However, alkaloids, mucilages, anthocyanins and free quinones are absent from the leaf extract.

Table 1: Results of phytochemical screening of hydroethanol and aqueous extracts of *D. guineense*

Family of chemical compound researched	Hydroethanol extract
Flavonoids	+
Alkaloids	-
Tannins	+
Terpenoids	+
Mucilages	+
Reducing compound	+
Saponosides	+
Coumarins	+
Anthraquinones	+
Anthocyanins	-
Free quinones	-

(+) = Positive reaction
(-) = Negative reaction

3.2 Acute oral toxicity: The dose of 5000 mg/kg did not cause mortality or clinical signs of acute toxicity in rats observed over a short period of 48 hours and a long period (14 days). No organ abnormalities were observed at necropsy.

3.3 28-days subchronic oral toxicity

3.3.1 General behavior and mortality

Oral administration of the hydroethanol leaf extract of *D. guineense* for 28 consecutive days did not induce any obvious symptoms of toxicity in rats, even at the highest dose tested (600 mg/kg body weight per day). No lethality was recorded during the 28 days of extract administration. No differences in general behavior, food and water consumption were observed between rat groups.

3.3.2 Effect of hydroethanol leaf extract of *D. guineense* on body weight of rats

At doses of 300 mg/kg and 600 mg/kg, the hydroethanol extract did not show any significant effect ($p<0.05$) on the body weight of treated rats as compared to control animals (Figure 1). In the first week, there was a remarkable increase in the weight of the rats whatever the dose of extract administered (300 mg/kg and 600 mg/kg). The same was true for rats fed only distilled water (controls). At the end of the treatment, there was no significant difference in weight gain between treated and control rats.

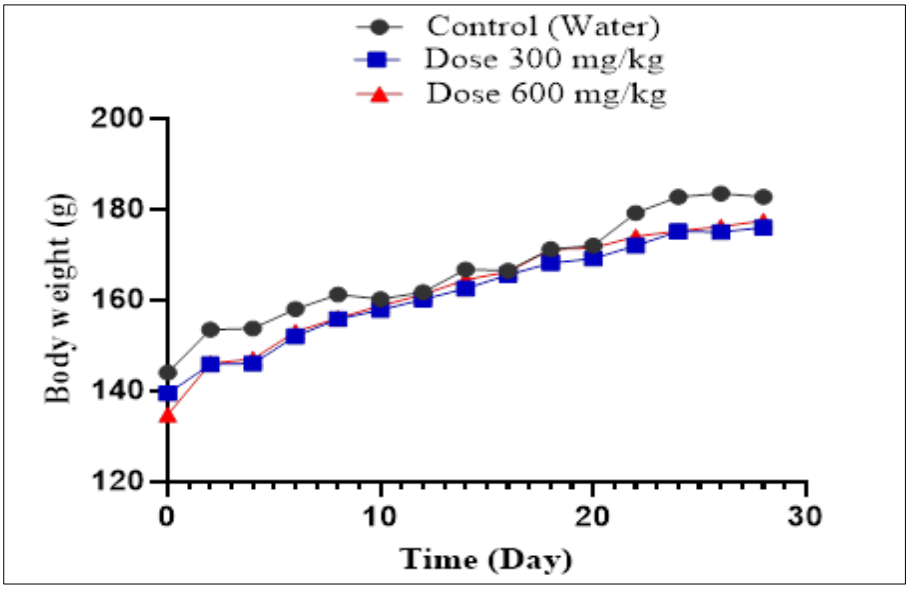


Fig 1: Effects of hydroethanol leaf extract of *Dichapetalum guineense* on weight gain in rats

3.3.3 Effects of hydroethanol leaf extract of *Dichapetalum guineens* on relative organ weight

The relative weight of an organ provides information on the evolution of the organ in relation to that of the whole organism. At the end of the four (4) weeks treatment with hydroethanolic leaf extract (HEE) of *D. guineense*, the relative weights of the organs did not vary significantly ($p<0.05$) compared to those of the controls (Table 2) and whatever the dose administered during the experiment.

Table 2: Effect of hydroethanol leaf extract (HEE) of *D. guineense* on relative organ weights in rats.

Organs	Control	Doses of *D. guineense* HEE	
		300 mg/kg	600 mg/kg
heart	0.648±0.031	0,583±0.020	0,606±0.044
liver	6,422±0,650	5,925±0,521	5,505±0,264
Rate	0,640±0,053	0,618±0,081	0,620±0,068
kidneys	0,998±0,054	0,899±0,023	0,949±0,074
lungs	1,111±0,078	1,080±0,11	1,067±0,089

Values are expressed as mean±MSE (N=5), No significant difference between control and treated groups ($p<0.05$), (ANOVA one way followed by Tukey's multiple comparison test).

3.3.4 Effect of hydroethanol leaf extract of *D. guineense* on biochemical parameters

Table 3 presents the effects of hydroethanol extract of *D. guineense* leaves (300 mg/kg and 600 mg/kg) on biochemical parameters in rats. Results show that oral administration of the extract for 28 consecutive days did not show any pronounced variation on biochemical parameters in treated rats. The onlyexceptions are blood glucose and total

cholesterol which varied in treated animals. The latter decreased significantly (p>0.05) at 600 mg/kg in comparision with control animals. On the other hand, alkaline phosphatase increased non-significantly (p>0.05) as compared to control rats at 600 mg/kg. In addition, parameters such as triglycerides, total bilirubin and urea showed a slight non-significant decrease ($P>0.05$) as compared to control rats at all doses (300 mg/kg and 600 mg/kg).

Table 3: Effect of hydroethanol leaf extract of *D. guineense* on biochemical parameters

Biochemical parameters	Control	Doses of *D. guineense* HEE	
		300 mg/kg	600 mg/kg
Triglycerides (g/L)	0.572±0.079	0.538±0.123	0.485±0.109
Blood glucose (g/dL)	0.600±0,062	0.740±0.026	0.823±0.020**
Total cholesterol (g/L)	0.658±0.031	0.682±0.039	0.510±0.024**
Total protein (g/L)	73.520±1.661	73.960±1.966	72.100±0.609
Creatinine (mg/L)	4.012±0.121	4.208±0.203	3.558±0.254
Total bilirubin (mg/L)	1.066±0.124	0.962±0.099	0.865±0.102
Direct bilirubin (mg/L)	0.836±0.081	0.840±0.081	0.765±0.103
Urea (g/L)	0.346±0.116	0.320±0.054	0.259±0.038
ALT (IU/L)	45.180±3.954	50.480±7.345	39.650±1.510
AST (IU/L)	168.700±20.96	192.900±16.110	165.100±5.435
PAL (IU/L)	143.600±6.323	144.700±20.490	200.100±19.700

Values are expressed as mean±MES, N=5 ; *p<0.05; ** p<0.01 significant difference as compared to controls (one-way ANOVA followed by Tukey's multiple comparison test), ASAT: Aspartate Aminotransferase; ALAT: Alanine Aminotransferase; PAL: Alkaline Phosphatase.

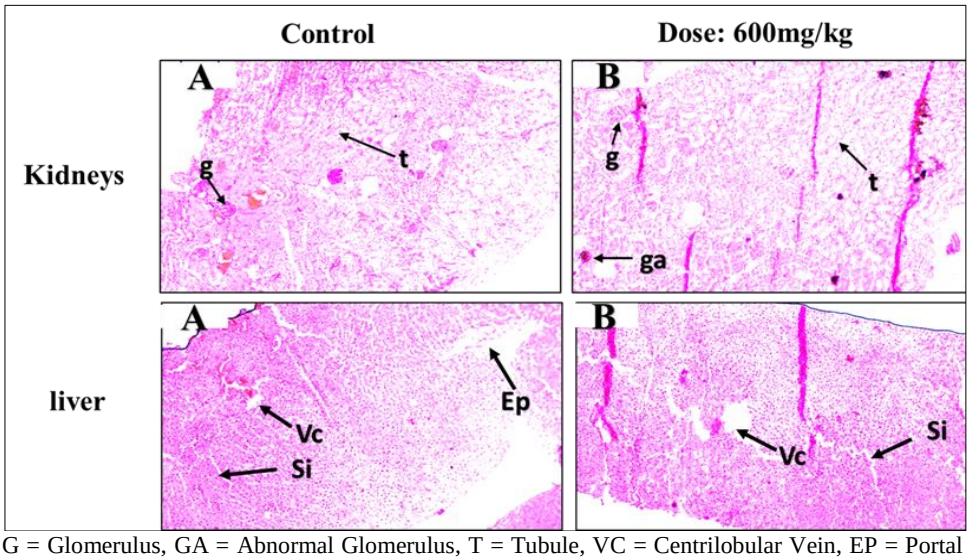
3.3.5 Effect of *D. guineense* leaf extract on haematological parameters in rats

Haematological analysis showed no significant changes in haematological parameters (Table 4). However, there was a non-significant increase (p>0.05) in white and red blood cells counts, haemoglobin (Hb) and mean corpuscular haemoglobin content in treated groups compared with the control group (Table 3). On the other hand, red blood cells count and the haematocrit (HTC) increased slightly in rats treated with 300 mg/kg and 600 mg/kg but this increase was not significant (p>0.05). At the same doses, the mean corpuscular haemoglobin concentration decreased non-significantly (p>0.05). Platelet count (PLT) decreased significantly (p<0.01) at 600 mg/kg.

Table 4: Effect of hydroethanol extract of *D. guineense* leaves on haematological parameter levels

Blood parameters	Control	Doses of *D. guineense* HEE	
		300 mg/kg	600 mg/kg
White blood cells (10^9/L)	13.270±2.400	17.33±2.835	16.28±1.627
Red blood cells (10^{12}/L)	7.938±0.520	8.232±0.085	8.308±0.120
Haemoglobin level (g/dL)	14.240±0.408	14.660±0.294	14.50±0.219
Haematocrit : HTC (%)	41.42±1.216	42.88±1.011	43.08±0.737
GMV (fL)	52.74±2.206	52.12±1.359	51.68±0.858
MCHT (pg)	18.14±0.729	17.78±0.404	17.66±0.233
CCMH (g/dL)	34.38±0.166	34.16±0.116	33.58±0.114
PLT (10^3/ µL)	1022±35.80	1118.±22.32	814±68.45**

Values are expressed as mean±MSE, N=5 ; *p<0.05; **p<0.01 (ANOVA one way followed by Tukey's multiple comparison test); Hb: Haemoglobin Level; HCT: Haematocrit Level; GMV: Mean Corpuscular Volume; MCHT: Mean Corpuscular Haemoglobin Content; MCHC: Mean Corpuscular Haemoglobin Concentration; PLT: platelet



G = Glomerulus, GA = Abnormal Glomerulus, T = Tubule, VC = Centrilobular Vein, EP = Portal Space, Si = Sinusoids.

Fig 2: Histological sections of kidneys and liver

3.3.6 Effects of hydroethanolic leaf extract of *D. guineense* on histological changes in rat: Sections (Figure 2) show normal architecture of the liver (a well-distinct centrilobular vein and hepatocytes) and kidney. No signs of tissue toxicity were observed in the liver. In the kidneys, an abnormal glomerulus (ga) was observed.

4. Discussion

The phytochemical study carried out showed that the extract of *Dichapetalum guineens* leaves from the Togolese flora contains major chemical groups, namely: flavonoids, anthraquinones, tannins, reducing compounds, mucilages, terpenes, saponosides and coumarins. However, the tests did not reveal the presence of free alkaloids, anthocyanins and quinones in the extract (Table 1). The richness of *D. guineense* species in these active secondary metabolites might therefore explain the traditional use of this plant to treat numerous diseases such as malaria [6] and diabetes in pregnant women [8]. Other authors have also shown in their studies that the different types of chemical compounds found in the hydroethanol extract of this plant have proven therapeutic effects [14, 15]. Comparing our results to those of Kakpo *et al.* [16], we note that they detected the presence of alkaloids in *D. guineense* leaves, while coumarins and terpenoids were absent. This difference is thought to be due to the nature of the soil, the climate and the harvesting period of the plant material [15, 17].

Acute oral toxicity study of the hydroethanol extract of *D. guineens* leaves revealed no particular signs of toxicity during the 14 days of observation. No rats died and there were no significant changes in behaviour during this period. In addition, macroscopic examination of the various organs at necropsy on day 15 revealed no abnormalities in these organs. These results show that the median acute toxicity value or LD_{50} of each extract is greater than 5000 mg/kg. Previous studies have shown that any product with an oral LD_{50} greater than 5000 mg/kg can be considered practically non-toxic [18, 19]. Hence, the hydroethanol extract of *D. guineense* might be safe to use. Similar results have been obtained on other plants using the same toxicological study methods as those used in this study [20, 21].

As no toxic effects were observed during acute toxicity study, an additional study was conducted to assess, for 28 consecutive days, the subchronic toxicity of the hydroethanol extract of *D. guineense* in Wistar rats. Results show that daily administration of the hydroethanol extract (300 mg/kg and 600 mg/kg) for 28 days caused neither death nor clinical signs of toxicity in the animals. Significant reduction or change in body weight is à sensitive indicator of toxicity after exposure to toxic substances (drugs, bioactive substances) [22]. In this study, no significant change in body weight gain was observed in treated rats in as compared to the control animals (Figure 1) after daily treatment for 28 days ; suggesting that prolounged oral administration of the extract has no effect on the normal growth of rats. According to Ilboudo *et al.* [23], by interacting with the food consumed, tannins can lead to a reduction in the weight of treated rats. This is because tannins are phenolic compounds with the ability to forming indigestible complexes with nutrients in food matrices, or with proteins in the body such as digestive enzymes. It is the complex formed that leads to a drop in nutritional yield.

The results obtained (Figure 1) in the present study might suggest that tannins had no effect on nutritional yield, given that their presence is reported in Table 1. A significant change in the relative weights of internal organs is a relatively sensitive indicator in toxicity study after exposure to bioactive substances [22, 24]. In this study, it was found that there was no significant change in relative organ weights.

The role of the hepatocytes is to neutralise toxins, and the role of the kidney is to purify the blood and eliminate waste. Analysing the functionning of the liver and kidney is therefore very important in assessing the toxicity of drugs and plant extracts because they are necessary for the survival of an organism [24]. So haematological and biochemical analyses were carried out to assess the alterations in liver and kidney functions that could be caused by ingestion of the hydroethanolic extract of *D. guineense*.

Haematological parameters provide information on haematopoietic functions (assessment of myeloid lineage cells) [25] changes in these haematological parameters in terms of blood content are predictive of toxicity [22]. Repeated administration of the extract at different doses (300 mg/kg and 600 mg/kg) after 28 days showed a slight non-significant ($p>0.05$) increase in certain haematological parameters (Hb, PLT, HTC and WBC). This suggests a possible stimulatory effect on haematopoietic functions. A critical analysis of these results suggests that the hydroethanol extract of *D. guineense* could have an antimalarial effect, as indicated by ethnobotanical surveys carried out among the Togolese population [6]. A previous study showed that antimalarial plants have à stimulating effect on the haematopoietic and immune systems [26]. In addition, an increase in white blood cells cont indicates a strengthening of the body's defences [27, 28]. The concentration of platelets (PLT) in the blood increased non-significantly ($p>0.05$) at a dose of 300 mg/kg. However, it decreased significantly ($p<0.05$) at a dose of 600 mg/kg compared with the control group and the group receiving the lowest dose ($p<0.01$). The results obtained are disparate for the hydroethanol extract of *D. guineense* and do not allow for any discussion. The results also revealed a slight non-significant ($p>0.05$) increase in red blood cell (RBC) levels correlated with a non-significant ($p>0.05$) increase in haemoglobin (Hb) and haematocrit (HTC) levels for all doses administered. This suggests that the extract is not toxic for haematological parameters. The results obtained in this study show that most haematological parameters are normal for the groups treated with the extract, whatever the dose administered.

Renal dysfunction can be assessed by simultaneous measurements of urea, creatinine and uric acid [29, 30]. In this study, the changes (non-significant decrease ($p>0.05$) in plasma urea concentrations in rats treated with the extract at both doses alone do not indicate an alteration in renal function, since no significant difference was found between creatinine levels in treated and control rats. Similarly, the extract acted on triglycerides, reducing their serum level non-significantly ($p>0.05$) for all doses administered. The extract is thought to have reduced the metabolism of fatty acids and triglycerides. With regard to total bilirubin and direct bilirubin, the results obtained were disparate for the hydroethanol extract of *D. guineense.* However, the difference compared to the control was not significant. In addition, the extract induced an increase in glycaemia at the end of treatment for all doses administered. This increase in blood glucose was significant ($p<001$) in rats treated with the 600 mg/kg dose. This result is not in agreement with that of Klotoé *et al.*, [8], who showed that the plant is used to treat diabetes in pregnant women in Benin. This difference is due to the fact that our extract (Togolese species) contains no alkaloids, whereas the Beninese species does [16]. It has been

shown that the presence of alkaloids in an extract can lower blood glucose levels [31].

Alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST) are serum marker enzymes associated with indices of health. They are usually used to diagnose and assess health status or signs of toxicity, particularly liver damage. In addition, an increase in serum LAP concentration is indicative of biliary tract obstruction. In this study, the extract did not induce any significant change ($p>0.05$) in transaminase (ASAT and ALT) and PAL levels in treated rats compared with control rats, regardless of the dose administered (300 mg/kg and 600 mg/kg). These non-significant changes in ASAT and ALT levels show that the hydroethanol extract of *D. guineens* does not have a hepatotoxic effect and that the extract did not induce toxic effects on cardiac tissue (Table 2 and Figure 2).

The overall results obtained for the two parameters studied are in line with the histological study carried out, with a slight alteration in the renal structure at the highest dose (Figure 2). The presence of a few abnormal glomeruli in the kidney tissue indicates that the extract has a slightly toxic effect on kidney tissue. In terms of liver tissue architecture, apart from dilatation of the centrilobular vein, the extract did not cause any structural damage to liver tissue. The use of this extract at this dose does not present any real toxicity for the two organs, the liver and the kidneys.

5. Conclusion

Secondary metabolites were identified in the hydroethanol extract of *D.guineens* in order to contribute to a better valorisation of the plant. The extract is not toxic at a single dose of 5000 mg/kg body weight. Its toxicity threshold indicates that the toxicity is slight. In addition, it did not induce any real changes in biochemical parameters in the rats treated. This reassures us that the extract has no toxic effect on the liver, kidneys or pancreas. In terms of haematological parameters, the extract induced an increase in blood sugar levels in animals treated with different doses over a long period.

Overall, the toxicological results obtained support the moderate safety of use of the hydroethanol extract at the therapeutic dose over both short and long periods.

6. Acknowledgement

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7. Conflicts of Interest

There are no conflicts of interest regarding this manuscript.

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