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Acute and Subacute Toxicity of *Annona senegalensis* Pers. and *Annona muricata* L. (annonaceae) and their Effects on Biochemical and Haematological Parameters on Wistar Rats

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Abstract

Introduction: The *Annonaceae* are generally found in the wooded savannahs of Africa and of great pharmacological importance. They are used for their antiseptic, anti-inflammatory, anti-convulsant and healing properties.

In order to establish the safety of these treatments, acute and sub-acute toxicity tests of hydroalcoholic extracts of *Annona senegalensis* and *Annona muricata* leaves on Wistar rats were performed. For the lethal dose (LD_{50}), which is the quantity of plant extract, administered at one time, that causes the death of 50% of the animals tested, LD_{50} was determined in Wistar rats, as well as the subacute toxicity after repeated administration for 28 days of doses of 500, 1000 and 2000 mg/kg body weight of the hydroalcoholic extracts of the leaves of *A. senegalensis* and *A. muricata*. Haematological and biochemical parameters were determined and histological examinations were performed at the end of the study.

Result and Discussion: For acute toxicity, no deaths were obtained which would place the LD_{50} at a dose above 5000 mg/kg for both plant extracts. The evaluation of subacute toxicity showed that the extracts had no significant effect on plasma and urinary biochemical parameters. However, analysis of haematological parameters revealed an increase in the number of white blood cells in females treated with the *A. muricata* extract, which was not the case for females treated with the *A. senegalensis* extract at doses of 1000 and 2000 mg/kg, in which a decrease in the number of red blood cells was observed, accompanied by an increase in the thrombocytic lineage at the 2000 mg/kg dose. However, males showed no significant change in haematological parameters compared to the control group.

The sinusoidal stasis noted with *A. senegalensis* extract on the liver and the lesions observed with *A. muricata* extract on the kidneys were more inflammatory than toxic in nature.

Conclusion: The hydroalcoholic extracts of the leaves of *Annona muricata* and *Annona senegalensis* thus studied did not show acute and subacute toxicity in Wistar rats by the oral route.

Introduction

Medicinal plants are used worldwide for the treatment and prevention of various diseases in both developed and developing countries [1,2]. These plants are increasingly indispensable in the lives of humans and animals, as they are used on a daily basis as a source of food (fresh fruits, vegetables, grains, seeds and nuts), for health care, for their aesthetic values, but also for ecological monitoring [3]. For this reason, the use of natural products has improved considerably over the years, as has research into these plants. In addition, people in these countries believe that natural products do not have toxic effects from their use [4,5]. Furthermore, herbal medicines are considered by many to be safer than synthetic medicines, as the phytochemicals contained in the extract of these plants act primarily at the level of the mechanisms of biochemical pathways [2].

The *Annonaceae* are plants that belong to the primitive dicotyledonous family that can be found as trees, shrubs or lianas in tropical areas. It comprises about two thousand species divided into about a hundred genera, and is represented in Senegal by the genera *Hexalobus* and *Annona*, which are very common in the Sudanian forests [6].

Annonaceae have the distinction of being commonly used in natural products. They have shown antibacterial, antifungal and antiprotozoal effects and are used in the treatment of diseases such as skin disease, intestinal worms, eye inflammation, HIV and cancer. In this family, molecules derived from shikimic metabolism are mainly found, with the presence of benzylisoquinoline alkaloids. Specific chemotaxonomic markers of this family are polycarpol and particular polyacetic derivatives such as acetogenins. Other metabolites are also isolated from *Annonaceae* (terpenes, tannins, cyclopeptides...).

Annonaceae are of great socio-economic and pharmacological importance and their fleshy fruits are edible [7]. Some species with fragrant flowers are used in perfumery and cosmetology (*Cananga odorata* or "Ylang Ylang") and others are used in certain regions as condiments (*Xylopia aethiopica*, "Ethiopian pepper") [8]. Their leaves, barks and roots are used for their medicinal properties. They are known to have antiseptic, anti-inflammatory, anti-convulsant and healing properties.

However, in recent years, the toxicity of herbal medicine has been proven, especially in terms of renal, cardiac and hepatic damage [9].

In order to secure the use of plants of the *Annona* genus, we proposed to study the acute and subacute toxicity of hydroalcoholic extracts of *Annona senegalensis* and *Annona muricata* on Wistar rats.

Materials and Methods

Plant materials

The plant materials used were fresh leaves of *Annona senegalensis* and *Annona muricata* collected respectively in the Niayes area of the Dakar region, and in the commune of Pout, located in the Thiès region.

The identification of these leaves was carried out in the Pharmacognosy and Botany Laboratory of the Faculty of Medicine, Pharmacy and Odontology (FMPO) of the Cheikh Anta Diop University of Dakar (UCAD) according to their botanical characteristics.

Animals

Wistar albino rats aged eight weeks, weighing between 110 and 190 g from the animal house of the UCAD Toxicology and Hydrology Laboratory were used for this experiment. They were acclimatised one

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week before the experiment at a temperature of 25°C and on a 12-hour day/night cycle. They had free access to clean tap water and a standard rat diet. They were kept in plastic cages lined with wood shavings. Food and water consumption was measured daily.

Prior to the start of the extracts, the rats were deprived of food, but fed water overnight and weighed.

Preparation of extracts

Freshly harvested leaves of *A. senegalensis* and *A. muricata* were washed, rinsed and dried in the pharmacology laboratory of the Faculty of Medicine, Cheikh Anta Diop University, Dakar. They were placed in the shade at room temperature (25°C) for 4 weeks before being ground separately.

After grinding, seventy-five grams (75 g) of *A. senegalensis* leaf powder were brought to a moderate boil (60°C) in 1 litre of ethanol/water mixture for 30 min distilled (8:2 v/v). In order to keep the volume constant and to renew the solvent, the vapours from the boiling were recovered using a condenser connected to a refrigerator so that the circuit could be kept closed. The extract obtained was then filtered and concentrated by evaporation using a rotavapor, until a pasty extract was obtained, which would be completely dried in a desiccator to obtain a dry extract.

The same operation was repeated for the preparation of the *A. muricata* leaf extract. Each of the extracts was administered by gavage to the different rats in the study.

After these different steps, the extraction yield was determined using the following formula:

$$Y = \frac{A}{B} x100$$

Where Y= extraction yield; A= mass of the residue; B= mass of the plant powder

Phytochemical study

The phytochemical study of the different leaves was carried out by characterisation reactions in tubes for the hydroalcoholic extract of *A. muricata* leaves according to the method describe by Bento E et al 2018 [10], and by thin layer chromatography (TLC) for the hydroalcoholic extract of *A. senegalensis* leaves according to the method describe by Sana N et al. 2012 [11].

Acute toxicity study

The determination of the lethal dose 50 (LD_{50}) for *A. senegalensis* extract was performed in accordance with the Organisation for Economic Co-operation and Development (OECD) guideline 425 [12]. A test limited to 5000 mg/kg body weight of hydroalcoholic extract of the leaves of *A. senegalensis* was administered to 2 groups of 3 fasting female rats in a single dose by gavage.

For *A. muricata* extract, 4 batches of 6 rats each (3 males and 3 females) were administered by gavage in single doses of 0, 500, 2000 and 5000 mg/kg body weight according to OECD guideline 401 [13]. After administration of the extracts to the different batches, the rats were observed for the first five hours and then daily for 14 days to note any signs of toxicity. At the end of the observation period the animals were sacrificed and the organs (liver and kidneys) were removed and washed with 0.9% NaCl before being preserved in 10% buffered formalin for histopathological examination.

Subacute toxicity study

The subacute toxicity study of each of the hydroalcoholic extracts required 4 batches of 6 rats, 3 male and 3 female rats randomly assigned to cages. The rats in the first batch, representing the control batch, were given distilled water. The other 3 batches received 500, 1000 and 2000 mg/kg body weight of hydroalcoholic extract of *A. muricata* and *A. senegalensis* respectively, orally for 28 days. This was carried out in accordance with OECD Guideline 407 [14]. Body weight and urine samples were taken weekly before gavage.

Urine sample collection: At the end of each week, the animals were placed individually in metabolism cages for 24 h. During these 24 h, urine was collected in vials for analysis of urinary biochemical parameters (glucose, protein, ketones) using U-AQS 3 GK® test strips. The reading and interpretation were done one minute after the strips were inserted into the urine fluid. Each colour was assigned a level indicated on the test kit packaging.

Haematological and biochemical assays: Blood from the rats was collected by cardiac puncture at the end of the experiment and divided into dry tubes for biochemical assays and EDTA tubes for haematological assays, respectively. The blood from the dry tubes was centrifuged at 3000 rpm for 5 min using the Biosystems A15 machine and the biochemical parameters were determined from the serum. Blood glucose was determined by the glucose oxidase method and glutamic oxaloacetic and pyruvic transaminases (ASAT and ALAT) were determined by the kinetic method [15,16]. Urea and creatinine were determined by the colorimetric method [17].

The haematological assays were carried out using the Coulter, which identifies the number of pulses by which the number of cells and the amplitude of the pulse produced are determined. The pulse produced is proportional in this case to the volume of the cell. This method allows the construction of erythrocyte, leukocyte and platelet histograms and the determination of the associated parameters [18,19].

Histopathological examination of the liver and kidneys was carried out using the standard haematoxylin/eosin colouring [20]. This method was used to determine the presence or absence of lesions associated with toxicity in the organs examined, after macroscopic examination of the various organs.

Statistical analysis

The values were presented as mean ± SEM (standard error of the mean). The results of the rat weights were analysed using Student's t-test, while the results of the biological parameters were analysed by ANOVA with Tukey's post-hoc test using R software. The difference was considered statistically significant for a probability level p less than 0.05.

Results

Extraction yields

According to formula (1), the yield of hydroalcoholic extraction of *Annona muricata* and *Annona senegalensis* leaves was 11.06 and 19.28% respectively (Table 1).

Phytochemical results

The chemical screening of the two extracts revealed the presence of all the chemical groups searched for with the exception of cardiotonic and anthracene heterosides (Table 2).

Results of toxicological studies

Acute toxicity: No deaths or signs of toxicity were observed throughout the acute study. After sacrifice, macroscopic observation of the organs revealed no abnormalities.

Table 1: Extraction yields of hydroalcoholic extracts of *A. senegalensis* and *A. muricata.*

	Hydroalcoholic extract of *A. senegalensis*	**Hydroalcoholic extract of *A. muricata***
Weight (g)	14,46	8,30
Yield (%)	19,28	11,06

Table 2: Summary of the chemical constituents present in the extracts.

Chemical groups	Extract of *A. senegalensis*	Extract of *A. muricata*
Flavonoids	++	+
Tannins	+++	+
Cardiotonic heterosides	ND	–
Anthracenes	ND	–
Alkaloids	+	+
Sterols and triterpenes	+	ND
Saponosides	ND	+

+ : positive; the number of "+" depends on the intensity of the staining; - negative; **ND** : not determined

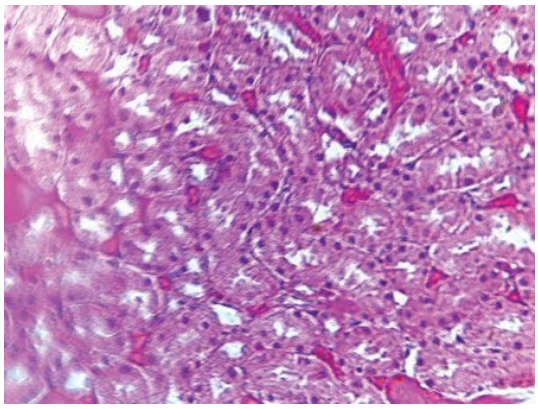


Figure 1: Section of kidney with normal histological appearance (HE*40) treated with *A. senegalensis* extract.

Microscopic examination of the kidneys of the control rats and of the six rats treated with *A. senegalensis* extract revealed no apparent lesions (Figure 1). In contrast, four of the rats treated with *A. senegalensis* extract showed rare megakaryocytes with blood stasis in the liver (Figure 2).

With *A. muricata* extract, one of the control rats showed simultaneous pyelitis and moderate subacute interstitial nephritis in the kidneys. In rats treated at 500 mg/kg, mild subacute cholangiohepatitis and rare inflammatory foci were noted in two rats and mild subacute pyelonephritis in one rat.

At 2000 mg/kg, minimal subacute cholangitis was noted in two of six rats, and minimal nonsuppurative interstitial nephritis in one of six treated rats. In contrast, at 5000 mg/kg, minimal subacute cholangitis was observed in one rat, and moderate and mild subacute pyelitis in two of the rats treated with *A. muricata* extract. 88.89% (8/9) of the rats with an affected organ were female.

Subacute toxicity: In the experiment, three male rats treated at 500 and 2000 mg/kg with *A. muricata* extract and one male rat treated with *A. senegalensis* extract at a dose of 2000 mg/kg died within minutes after gavage. Histopathological examination of the lungs of these rats revealed pneumonia due to false swallowing (Figure 3).

The results of the subacute toxicity showed no statistically significant difference in the weight changes of the control lot compared to all male rats treated with both *A. senegalensis* and A. *muricata* extracts (Figure 4 and 5) ($P > 0.05$). In contrast, in female rats, a significant increase in body weight was observed in female rats treated with 2000 mg/kg of *A. muricata* extract at D21 and D28 (Figure 6) (P

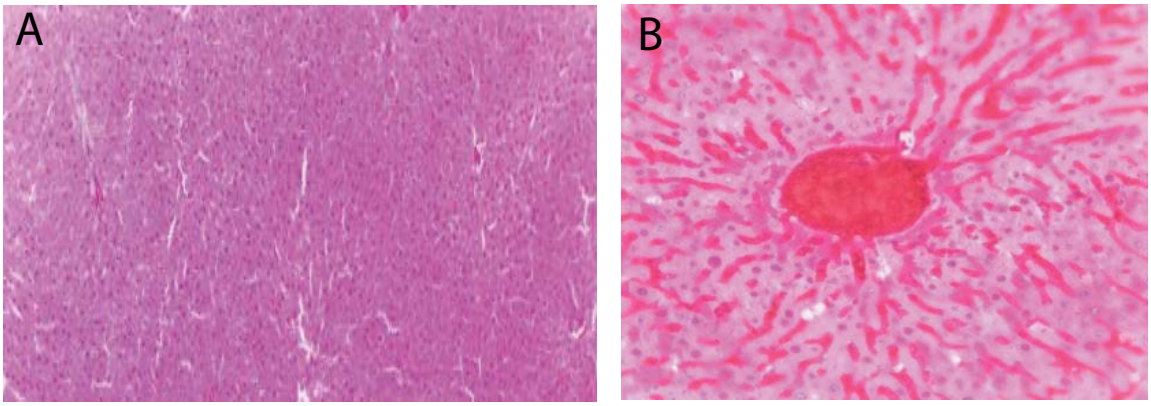


Figure 2: Sections of liver with normal histological appearance (HE*10) (A) and sinusoidal stasis (HE*40) (B) treated with hydroalcoholic extract of *Annona senegalensis* leaves.

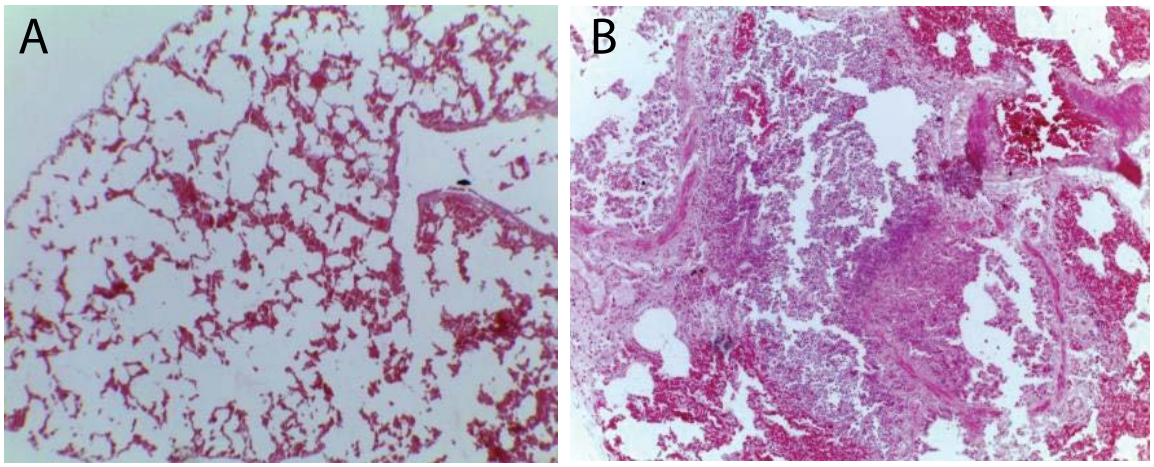


Figure 3: Sections of lungs with normal histological appearance (HE*40) (A) and severe suppurative bronchopneumonia (HE*10) related to false swallowing (B).

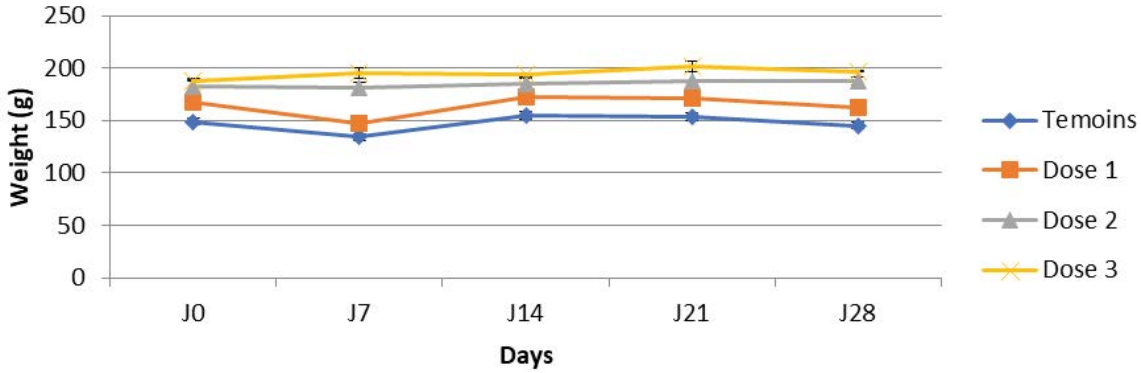
< 0.05). Significant weight gain was also noted in rats treated with *A. senegalensis* extract at 500 mg/kg at the end of the experiment and at 1000 and 2000 mg/kg at D21 (Figure 7).

The search for albumin, sugar and ketone bodies in the urine did not show the presence of these compounds in this biological medium.

With the exception of the increase in enzymatic activity and the significant difference in aspartame aminotransferase (ASAT) values between the control lot and the lot that received the *A. muricata* extract at the dose of 2000 mg/kg, the hydroalcoholic extracts of the leaves of *A. senegalensis* and *A. muricata* had no significant effect on the biochemical and haematological parameters of male rats (Tables 3, 4, 5 and 6).

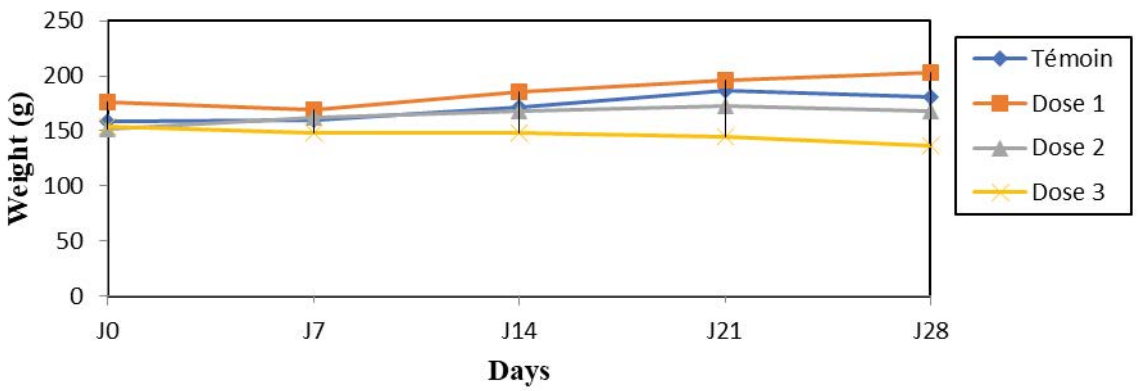
On the other hand, the observations made between the control and the different other batches, in the subacute toxicity study revealed a decrease in blood glucose and an increase in alanine-amino transferase (ALAT) activities (Table 7) in female rats treated with *A. senegalensis* extract at the dose of 500 mg/kg. Statistical analysis also revealed a slight decrease in red blood cells at 1000 and 2000 mg/kg and an increase in platelets only at 2000 mg/kg (Table 8), with significant differences from the control lot.

In female rats treated with *A. muricata* extract, there was an increase in ASAT activity at the lowest dose (500 mg/kg) and at the highest dose (2000 mg/kg), but also an increase in creatinine concentrations at 500 and 1000 mg/kg (Table 9). In addition, there was an increase in haematocrit percentage at the highest dose (2000 mg/kg) and a significant increase in white blood cell count at all doses tested (Table 10).



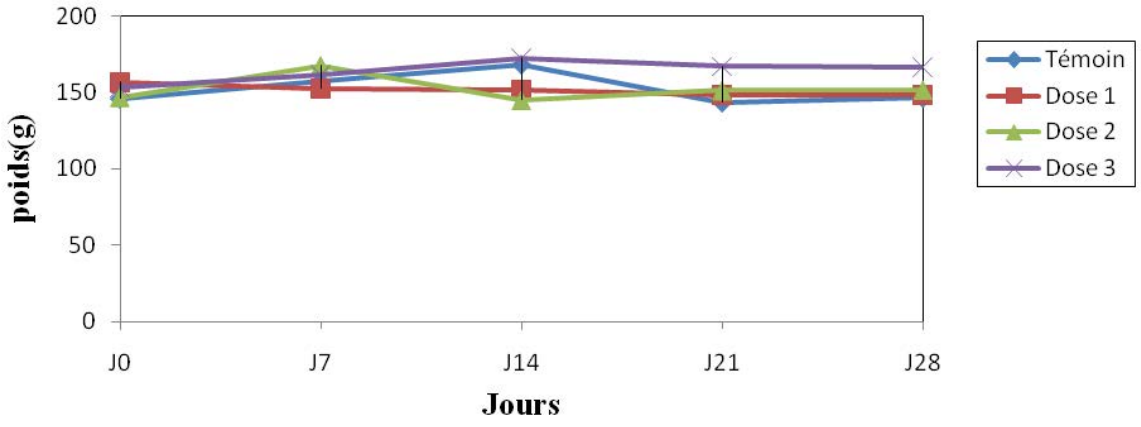
Results are expressed as a mean. Dose 1=500 mg/kg; Dose 2=1000 mg/kg; Dose 3=2000 mg/kg

Figure 4: Weight evolution of male rats treated with *A. senegalensis* extract from D0 to D28.



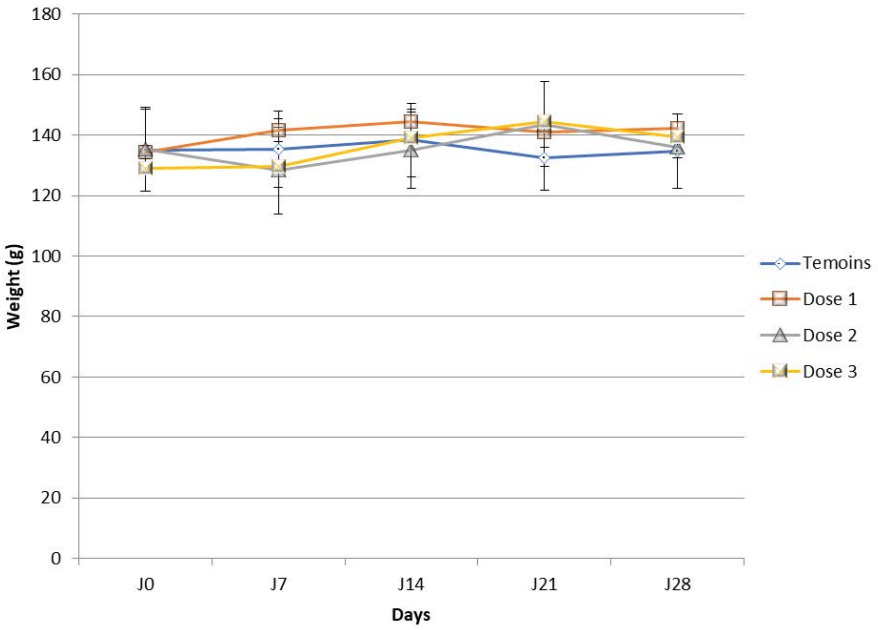
Results are expressed as a mean. Dose 1=500 mg/kg; Dose 2=1000 mg/kg; Dose 3=2000 mg/kg

Figure 5: Weight evolution of male rats treated with *A. muricata* extract from D0 to D28.



Results are expressed as a mean. Dose 1=500 mg/kg; Dose 2=1000 mg/kg; Dose 3=2000 mg/kg

Figure 6: Weight evolution of female rats treated with *A. muricata* extract from D0 to D28.



Results are expressed as a mean. Dose 1=500 mg/kg; Dose 2=1000 mg/kg; Dose 3=2000 mg/kg

Figure 7: Weight evolution of female rats treated with *A. senegalensis* extract from D0 to D28

Table 3: Biochemical parameters in male rats treated with hydroalcoholic extract of *Annona senegalensis* leaves.

Parameters	Control n=3	Treated 500 mg/kg n=3	Treated 1000 mg/kg n=3	Treated 2000 mg/kg n=2
Blood glucose (g/l)	0.77 ± 0.08	0.74 ± 0.11	0.80 ± 0.03	0.99 ± 0.11
Urea (mg/l)	72.66 ± 6.98	57.66 ± 1.20	69.33 ± 4.40	75.33 ± 3.7
Creatinine (g/l)	0.60 ± 0.03	0.77 ± 0.13	0.68 ± 0.04	0.67 ± 0.05
ASAT (IU/l)	225.66 ± 19.32	331.33 ± 56.48	266 ± 37.07	260.93 ± 34.04
ALAT (IU/l)	117.33 ± 4.33	132 ± 10.50	122 ± 0	108 ± 18.45

Results expressed as mean ± standard error of the mean ; n = number of rats

Table 4: Haematological parameters in male rats treated with hydroalcoholic extract of *Annona senegalensis* leavesmean.

Parameters	Control n=3	Treated 500 mg/kg n=3	Treated1000 mg/kg n=3	Treated 2000 mg/kg n=2
RBC (10^6/mm^3)	8.55 ± 0.12	8.89 ± 0.2	8.22 ± 0.15	8.47 ± 0.47
WBC (10^3/mm^3)	7.69 ± 0.03	7.725 ± 1.62	7.88 ± 1.08	5.68 ± 0.55
PL (10^3/mm^3)	521 ± 32	687 ± 16	625.5 ± 4.5	456.5 ± 266
Hb (g/dl)	16.25 ± 0.35	16.8	15.6 ± 0.6	15.55 ± 0.45
Ht (%)	46.5 ± 1.5	47.6 ± 0.7	44.75 ± 1.15	45.85 ± 1.65

Results expressed as mean ± standard error of the mean ; n = number of rats

Table 5: Biochemical parameters in male rats treated with hydroalcoholic extract of *Annona muricata* leaves.

Parameters	Control n=3	Treated 500 mg/kg n=1	Treated 1000 mg/kg n=3	Treated 2000 mg/kg n=2
Blood glucose (g/l)	1.02 ± 0.42	1.01	0.85 ± 0.07	1.05 ± 0.19
Urea (g/l)	0.49 ± 0.04	0.66	0.51 ± 0.07	0.48 ± 0.14
Creatinine (mg/l)	6.74 ± 0.55	7.37	6.64 ± 0.52	6.37 ± 0.89
ASAT (IU/l)	188.12 ± 30.65	194.13	219.21 ± 22.14	307.31 ± 70.50*
ALAT (IU/l)	207.91 ± 47.18	263.17	185.47 ± 5.40	203.73 ± 18.47

Results expressed as mean ± standard error of the mean ; n = number of rats

* Statistically significant difference from control group ($p < 0.05$)

Table 6: Haematological parameters in male rats treated with hydroalcoholic extract of *Annona muricata* leaves.

Parameters	Control n=3	Treated mg/kg n=1	Treated 1000 mg/kg n=3	Treated2000 mg/kg n=2
RBC (10^6/mm^3)	8.00 ± 0.20	6.49	7.93 ± 0.49	7.99 ± 0.48
WBC (10^3/mm^3)	14.8 ± 2.56	8.5	13.43 ± 1.19	14 ± 0.70
PL (10^3/mm^3)	786 ± 67.13	531	830.66 ± 19.29	762 ± 22.62
Hb (g/dl)	15.06 ± 0.50	11.5	15 ± 0.43	15.45 ± 0.35
Ht (%)	45.53 ± 1.84	34.8	44.66 ± 2.19	45.3 ± 1.97

Results expressed as mean ± standard error of the mean ; n = number of rats

Table 7: Biochemical parameters in female rats treated with hydroalcoholic extract of *Annona senegalensis* leaves.

Parameters	Control n=3	Treated 500 mg/kg n=3	Treated 1000 mg/kg n=3	Treated 2000 mg/kg n=3
Blood glucose (g/l)	0.89 ± 0.02	0.74 ± 0.03*	0.88 ± 0.04	0.97 ± 0.08
Urea (g/l)	68.66 ± 1.85	80.33 ± 8.19	71.5 ± 9.5	59.5 ± 14.5
Creatinine (mg/l)	0.61 ± 0.08	0.76 ± 0.07	0.71 ± 0.1	0.69 ± 0.03
ASAT (IU/l)	212 ± 26.50	282 ± 28.82	224.5 ± 4.5	229.33 ± 31.56
ALAT (IU/l)	111 ± 7	168 ± 2*	97 ± 8	104 ± 1

Results expressed as mean ± standard error of the mean ; n = number of rats
* Statistically significant difference from control group ($p < 0.05$)

Table 8: Haematological parameters in female rats treated with hydroalcoholic extract of *Annona senegalensis* leaves.

Parameters	Control n=3	Treated 500 mg/kg n=3	Treated 1000 mg/kg n=3	Treated 2000 mg/kg n=3
RBC (10^6/mm^3)	8.22 ± 0.19	7.9 ± 0.55	7.86 ± 0.18*	7.63 ± 0.16*
WBC (10^3/mm^3)	7.62 ± 0.49	8.55 ± 1.61	6.19 ± 0.3	8.79 ± 0.13
PL (10^3/mm^3)	602.5 ± 61.5	463 ± 28	600 ± 183	725 ± 56*
Hb (g/dl)	16.3 ± 0.4	15.55 ± 0.75	14.95 ± 0.05	14.75 ± 0.15
Ht (%)	47.85 ± 1.85	46.95 ± 1.65	45 ± 1.5	44.85 ± 0.05

Results expressed as mean ± standard error of the mean ; n = number of rats
* Statistically significant difference from control group ($p < 0.05$)

Table 9: Biochemical parameters in female rats treated with hydroalcoholic extract of *Annona muricata* leaves.

Parameters	Control n=2	Treated 500 mg/kg n=2	Treated 1000 mg/kg n=3	Treated 2000 mg/kg n=2
Blood glucose (g/l)	1.08 ± 0.13	1.12 ± 0.09	1.18 ± 0.32	1.00 ± 0.14
Urea (g/l)	0.46 ± 0.10	0.56 ± 0.15	0.55 ± 0.11	0.42 ± 0.02
Creatinine (mg/l)	5.69 ± 0.02	6.69 ± 0.27*	6.88 ± 0.40*	6.44 ± 0.61
ASAT (IU/l)	162.98 ± 0.91	242.65 ± 22.60*	166.52 ± 72.70	174.09 ± 1.38*
ALAT (IU/l)	137.51 ± 42.60	164.65 ± 28.73	195.06 ± 64.34	148.37 ± 29.74

Results expressed as mean ± standard error of the mean ; n = number of rats
* Statistically significant difference from control group ($p < 0.05$)

Table 10: Haematological parameters in female rats treated with hydroalcoholic extract of *Annona muricata* leaves.

Parameters	Control n=2	Treated 500 mg/kg n=2	Treated 1000 mg/kg n=3	Treated 2000 mg/kg n=2
RBC (10^6/mm^3)	6.22 ± 0.91	7.14 ± 0.10	6.53 ± 0.89	7.82 ± 0.08
WBC (10^3/mm^3)	8.15 ± 0.49	10.9 ± 1.13*	11.3 ± 1.74*	14.95 ± 5.16*
PL (10^3/mm^3)	871 ± 222.03	784 ± 250.31	754.33 ± 283.54	888 ± 4.24
Hb (g/dl)	12.35 ± 1.90	14.15 ± 1.20	12.96 ± 2.05	15.55 ± 0.21
Ht (%)	37.25 ± 5.58	42.75 ± 4.17	38.63 ± 4.95	45.95 ± 0.35*

Results expressed as mean ± standard error of the mean ; n = number of rats
* Statistically significant difference from control group ($p < 0.05$)

The results of histological analysis revealed no lesions in the livers and kidneys of rats treated with the hydroalcoholic extract of *A. senegalensis* leaves at all doses tested. In contrast, 42.86% of the organs (livers and kidneys) of rats treated with the hydroalcoholic extract of the leaves of *A. muricata* showed stasis lesions (minimal, mild and moderate sinusoidal stasis) and 52.38% showed minimal and mild subacute pyelitis and interstitial nephritis at all doses tested. 57.14% of the affected organs were in female rats.

Discussion

The leaves of *Annona senegalensis* and *Annona muricata* are used in traditional medicine in the treatment of various ailments and in particular as a sedative, anti-diarrheal, anti-infectious, antitussive, healing and anti-inflammatory [21]. The objective of the present study was to evaluate, in vivo, the acute and subacute toxicity of hydroalcoholic extracts of *A. senegalensis* and *A. muricata* leaves, with a view to securing its therapeutic use.

The hydroalcoholic extraction used in this study gave us an overall yield below 20%. This low yield may be due to the hydroalcoholic extraction method used [22]. The ultrasound-assisted extraction of phenolic compounds from the by-products and pulp of *Annona muricata* had already achieved a yield of 32-37%, higher than the conventional extraction method, which was 14-16% [23]. Although the yield may also depend on the feedstock as suggested by Aguilar-Hernández G et al 2019, the combination of several extraction techniques as described by Romero-Diaz R et al 2018 seems to be a possibility to obtain a better yield, which could contribute to reduce the extraction time while doubling the expected yield [24].

Phytochemical analysis of the extracts revealed high levels of flavonoids and tannins in the extracts of *A. senegalensis* and *A. muricata*. On the other hand, low contents of alkaloids, sterols and triterpenes were found in the extracts of *A. senegalensis*. These same compounds were found in the *A. muricata* extract, except for sterols and triterpenes which were not investigated. These similar results between the two extracts could justify the multiple uses made of them in traditional medicine [25].

The toxicity study allows the identification of functional and/or anatomopathological alterations that may be attributable to the administration of an active substance. Following the administration of the different extracts at different doses, the results obtained revealed no influence of the extracts on the body weight of male rats. However, in female rats, the administration of the extracts was correlated with an

increase in body weight between D21 and D28, compared to the control group. Thus, the administration of *A. senegalensis* and *A. muricata* extracts did not cause a decrease in body weight in the different groups compared to the control group. According to several authors, the increase in body weight after administration of a drug generally indicates the absence of toxicity, unlike the decrease in body weight which is an indication of the toxic effect of a substance [26-28].

The acute toxicity study of the two hydroalcoholic extracts indicates a relatively high safety profile with a lethal dose (LD_{50}) above 5000 mg/kg. This result places both hydroalcoholic extracts in category 5 of the OECD Globally Harmonised Classification System, and allows the extracts of *A. senegalensis* and *A. muricata* to be considered as relatively non-toxic by the oral route [29]. Several previous studies had already found similar results using other forms of extracts or a different experimental model [26,30-33]. The use of other forms of extracts such as methanolic extract and ethanolic extract seems not to influence the acute toxicity in rats. These authors also showed that oral administration of methanolic extracts of leaves and stem bark of *A. senegalensis* up to 5000 mg/kg did not result in any mortality or apparent signs of toxicity in either rats or rabbits [31]. On another hand, the study conducted by De Sousa et al. in 2010 showed that the ethanolic extract of the leaves of *A. muricata* had a toxic effect in mice treated orally at doses between 0.5 and 3 g/kg, for an LD_{50} of 1.67 g/kg. [34].

The total aqueous extract of *A. senegalensis* did not affect plasma and urine biochemical parameters compared to the control group. The normal values of urea, creatinine and the absence of glucose, ketone bodies or albumin in the urine suggest that *A. senegalensis* extract does not affect renal function. These observations were also made in rats given *A. muricata* extract. The increase of these values can be observed in case of alteration of the renal function [35].

Evaluation of haematological parameters revealed an increase in white blood cell count in females treated with *A. muricata* extract, which was not the case for females treated with *A. senegalensis* extract at 1000 and 2000 mg/kg, in which a decrease in red blood cell count was observed, accompanied by an increase in the thrombocytic lineage at 2000 mg/kg. However, males showed no significant variation in haematological parameters compared to the control group. This difference may be due to a variation in the immune response or a stress state in the rats, as the degree of immunological stimulation is one of the important factors responsible for the variation of certain haematological parameters, including circulating lymphocytes in the body. This suggests possible immune modulating effects of the extract in female rats [26,36]. Furthermore, the part of the plant used and the method of preparation of the extracts may also play a role in the variation of some parameters, which could explain the difference in observation compared to the results Okoye T.C et al. 2012 who had observed a significant increase in total leucocyte count after administration of methanol-methylene chloride extract of *A. senegalensis* root bark in rats [26,37].

The mean ALAT values, on the other hand, remained within normal limits in rats treated with *A. muricata* extract. However, the mean ASAT values at 2000 mg/kg in male rats and at 500 and 2000 mg/kg in female rats reflect probable cellular damage at the hepatic level [38]. The histological assay, however, did not show any abnormalities in the livers. It can therefore be concluded that the hydroalcoholic extract of the leaves of *A. muricata* did not cause any observable lesions in the livers.

With the *A. senegalensis* extract, the elevated ALAT values in female rats treated at 500 mg/kg were also not due to a toxic effect of the extract. The sinusoidal stasis noted with *A. senegalensis* extract on the liver and the lesions observed with *A. muricata* extract on the kidneys were more inflammatory than toxic in nature. This could be the cause of the variation in white blood cell count or creatinine at 500 and 1000 mg/kg as observed in female rats.

Conclusion

The hydroalcoholic extracts of *Annona muricata* and *Annona senegalensis* leaves studied did not show acute and subacute toxicity in Wistar rats by the oral route. Furthermore, the two extracts show several similarities which may justify their common pharmacological activities. The differences noted between the sexes at different doses may be both hormonal and physiological in nature. However, the presence of acetogenins and alkaloids, suspected of neurotoxicity, invites caution regarding regular and repeated use in cases of Parkinson's disease. A chronic toxicity study is thus envisaged to complete these results.

Conflicts of Interest

The authors declare that they had no conflicts of interest.

Declaration of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors Contributions

Awa NDONG: she played a substantial role in the drafting of the project, the work activities, the analysis and interpretation of the results, the drafting of the article and its proof-reading, corresponding to a significant contribution to the intellectual and scientific content of the article

Robert FAOMOWE FOKO: Participated in the interpretation of the results, the writing of the article and its proofreading

Fatoumata BAH: it has participated in a proofreading exercise corresponding to a significant contribution in terms of intellectual and scientific content

Jessica Carmelia MBEMBA PELEKA: it has participated in a proofreading exercise corresponding to a significant contribution in terms of intellectual and scientific content

Aminata TOURE: she played a substantial role in the design of the research project and protocol, participated in the writing of the article and carried out a review corresponding to a significant contribution to the intellectual and scientific content

Yaghouba KANE: He played a substantial role in the design of the project and the research protocol and in the analysis and interpretation of the results.

Guata Yoro SY: He played a substantial role in the design of the project and the research protocol and in the analysis and interpretation of the results.

Mamadou FALL: He supervised the whole work, coordinated the writing of the article and explicitly approved the final version of the manuscript and its scientific content.

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