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Evaluation of Hepatoprotective Activity and Acute Toxicity of Guiera Senegalensis Leaf Extract on Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MUH, EAT, CNF and BH conceived and designed the study and drafted manuscript. Authors NNB and MUH coordinated laboratory analysis and data assembly. All authors read and approved the final manuscript.

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ABSTRACT

A liver is an essential organ that performs several metabolic activities in body. Various chemicals as well as pathologies which affecting it result in harmful consequences. Management of liver pathologies involves expensive and even toxic therapeutic strategies. 80 % by populations in developing countries used medicinal plants which constituted a significant reservoir of molecules with diverse and various properties including hepatoprotective properties. To rectify these problems, *Guiera senegalensis* has been in existence that subjected to few studies and highlighted

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hepatoprotective properties, hence the interest of this study. Three extraction methods were carried out, infusion, decoction, maceration (aqueous, ethanolic). The hepatoprotective activity of the extracts was explored on 42 *Wistar* rats divided into 7 groups of 6 rats each. The groups including 4 test groups, were treated with the extract at 500 mg/kg of each extract. A healthy group that received distilled water (positive control group) were treated with paracetamol at 640mg/Kg. A reference group received a drug (Silymarin)
The rats were treated for 14 days. The rats were then sacrificed, blood sample were collected for biochemical analyses. Some organs were removed for histological analysis as well. The acute toxicity of the extract obtained by hydro-ethanolic maceration at a dose of 2000 mg/kg was conducted in accordance with OECD guideline 420. The study of the hepatoprotective activity of plant extracts showed a significant decrease in transaminase activities in the rats treated with the extract obtained by aqueous maceration, AP, a significant decrease in total proteins in the extract obtained by hydro-ethanolic maceration. It was revealed that, the increase in *in-vivo* antioxidants found to be high in those treated with the hydro-ethanolic extract. The hydro-ethanolic extract did not show any significant toxicity.

Keywords: Guiera senegalensis; hepatoprotection; leaf extract; wistar rats.

1. INTRODUCTION

The liver is one of the largest organs in mammals and is involved in numerous functions including homeostasis and metabolism of xenobiotics. Due to these functions, the liver is often subject to attacks such as microbial infections [1]. These microbial attacks can cause hepatitis, which is the inflammation of the liver. Hepatitis can also be caused by alcohol, immunity, medications, and toxins but most often by hepatitis viruses [2].

According to the WHO, hepatitis B and C cause the death of 1.1 million people per year and 3 million new infections. 10 % of people who suffer from hepatitis B are diagnosed and 22% receive treatment. For hepatitis C, 21% of people are diagnosed but 62% of these diagnosed people receive treatment [3].

Many natural medications exist whose main goal is to protect the liver against attacks. This is the case of hesperidin, which acts as an anti-inflammatory, chrysin protecting against hepatotoxicity induced by methotrexate by restoring cellular antioxidant defence. We also have quercetin, sylimarin, hyperoside, glycirrhizic acid. These molecules extracted from plants have demonstrated hepatoprotective properties [4]. The act of healing with plants has always been of great importance in the lives of men; even today 70 to 80% of the populations in developing countries use plant-based medicines [5]. We have as much proof on a religious level of the use of plants in human health; this is the case of the mention in the Holy Koran of the use of dates for health [5]. Traces in prehistory would indicate that plants used as spices were used as medicines. We are also observing a much more codified development of traditional medicine such as Ayurveda [6]. This is generating great interest in the development of plants based medicines.

Guiera senegalensis is a plant from the Combretaceae family, found in the Sahelian regions of West and Central Africa. It is used to treat abdominal pain, dysentery, rheumatism, constipation, diarrhea, jaundice. Studies have demonstrated its antioxidant, anti-inflammatory and antibiotic properties [7]. The present study will focus on the hepatoprotective property of *Guiera senegalensis* leaf extracts as well as its acute toxicity.

2. METHODOLOGY

2.1 Materials

2.1.1 Plant material

The study took place in the preclinical studies and toxicology laboratory of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé. The sample (*Guiera senegalensis* leaf) was collected at town of Guider, North Cameroon. The species (*Guiera senegalensis* leaf) was identified at the National Herbarium of Cameroon in comparison with herbarium specimen No°14902/SRF.cam by Mr. Nana Victor and treated as follows:

The *Guiera senegalensis* leaves were dried under shade and ground into power in an electric robot.

- Preparation of extracts: leaf extracts were prepared using four methods:
- The decoction: In this method, the water-leaf powder mixture was heated for 30

minutes in a water bath and cooled. Once cooled, the mixture was filtered [8].

- Maceration: Two different solvents: water and ethanol. 100g of *G. senegalensis* leaf powder were weighed and introduced into an Erlenmeyer flask. 300ml of solvent was added and the mixture was left at room temperature for 48 hours with regular stirring. Once the 48 hours had passed, the mixture was filtered in order to separate the waste [8].
- Infusion: it follows the same principle as maceration but in this case, the solvent used was water at it's boiling point (100C) [8].

2.1.2 Animal material

Wistar rats (*Rattus norvegicus*) with masses between 80-100g were used. They were raised in the animal facility of the Faculty of Medicine and Biomedical Sciences of the University of Yaounde I.

2.1.3 Distribution of animals

The protocol used was that proposed by Muhammad T et al. and modified [9]. 42 rats used, divided into 7 groups of 6 rats each. Group 1 (healthy control) received distilled water, group 2 (negative control) received 640 mg/Kg of paracetamol to induce hepatotoxicity; group 3 receives 500 mg/kg of extract obtained by hydro-ethanolic maceration; group 4 receives 500 mg/kg of extract obtained by aqueous maceration; group 5 received 500 mg/kg of extract obtained by infusion. Group 6 received 500 mg/kg of extract obtained by decoction; group 7 received silymarin at a dose of 300 mg/kg [10]. The rats were treated for 14 days. The Table 1, illustrates the distribution and treatment that each group of rats received during the study:

2.2 Evaluation of Zootechnical Parameters

2.2.1 Weight assessment

During the 14 days for the evaluation of the hepatoprotective activity of leaf extract, the rats were weighed every day in order to determine their masses. The starting mass being between 80-100g.

2.2.2 Assessment of water and food intake

During the 14 days of the study, the water intake of each group of rats was evaluated. The starting volume being 300ml, using a burette each day. Daily water consumption and food consumption computed.

2.3 Preparation of Solutions to Administer

2.3.1 Extracts solutions

According to the OECD guidelines N°420: Acute Oral Toxicity – Fixed Dose Procedure, which was applied in this study, each animal received not more than 2ml/100g concentration of extract and. the following formula was used to determine the concentration to be administered:

$$Volume\ to\ be\ administered(ml) = \frac{\left[dose\ \left(\frac{mg}{Kg}\right) x\ animal\ weight\ (Kg)\right]}{weight\ concentration\ (\frac{mg}{kg})}$$

Table 1. Distribution of the different groups of rats during the study

Groups	Treatment
Group 1	Healthy control, receives 2ml of distilled water
Group 2	Negative control, receives 640 mg/Kg of paracetamol at a dose of 2ml/100g body weight for 14 days
Group 3	Receives 500mg/kg of extract obtained by ethanolic maceration + 640 mg/Kg of paracetamol at a dose of 2ml/100g
Group 4	Receives 500mg/kg of extract obtained by aqueous maceration + 640 mg/Kg of paracetamol at a dose of 2ml/100g
Group 5	Receives 500mg/kg of extract obtained by infusion + 640 mg/Kg of paracetamol at a dose of 2ml/100g
Group 6	Receives 500mg/kg of extract obtained by decoction + 640 mg/Kg of paracetamol at a dose of 2ml/100g
Group 7	Positive control, receives silymarin at a dose of 300mg/kg + 640 mg/Kg of paracetamol at a dose of 2ml/100g

From this formula, we could therefore determine the mass of extract required (weight concentration) in order to administer in 1ml of solution:

$$weight\ concentration\ \left(\frac{mg}{ml}\right) = \frac{\left[Dose\ \left(\frac{mg}{Kg}\right) x\ animal\ weight(Kg)\right]}{Volume\ to\ be\ administred\ (ml)}$$

2.3.2 Preparation silymarin solution

The silymarin solution was prepared according to OECD rule. Therefore, to administer 300mg/Kg, 750mg of silymarin should be used. The specialty used here is Silybon® 140mg tablet. Which corresponds to six tablets to be crushed and dissolved in 25ml of distilled water

2.3.3 Preparation of paracetamol solution

The rats received 640 mg/Kg of paracetamol as proposed by Muhammad T et al [9]. The formula used to prepare the paracetamol solution to administer is as follows:

$$weight\ concentration\ \left(\frac{mg}{ml}\right) = \frac{\left[Dose\ \left(\frac{mg}{Kg}\right) x\ animal\ weight(Kg)\right]}{Volume\ to\ be\ administred\ (ml)}$$

2.3.4 Induction of hepatotoxicity

For the induction of hepatotoxicity the rats were treated with 2ml/100g body weight of paracetamol at 640 mg/Kg for 14 days. During this period the rats should be fed with a normal diet. On the 15th day, the rat were sacrificed. The blood, kidneys and livers were collected.

2.3.5 Assay of biochemical parameters

The serum and liver homogenate obtained from rats from different batches was used to determine the enzymatic activity of specific and non-specific biochemical parameters which are known to be modified by hepatotoxins, in order to evaluate the hepatoprotective activity of Guiera senegalensis.

2.3.6 Dosage of ALT, AST, AP, Creatinine, total proteins, HDL-cholesterol, triglycerides, uric acid, albumin, urea

The determination of liver and kidney function parameters such as AST [11], ALT [12], AP [13], Creatinine [14], total proteins [15], triglycerides [16], albumin [17], HDL-cholesterol [18], urea [19] were carried out using a commercial assay kit (Chronolab SYS S.L. Avenida diagonal 609, planta 10, DRU28 Barcelona. Spain). While quantification of biochemical parameters were carried out using a commercial assay kit (Chronolab SYS S.L. Avenida diagonal 609, planta 10, DRU28 Barcelona. Spain)

2.4 Dosage of MDA, SOD, Glutathione, Catalase

2.4.1 Malondialdehyde (MDA) dosage

Malondialdehyde is a carbonyl compound resulting from the decomposition of fatty acid hydroperoxides. It reacts with thibarbituric acid to give pink-coloured chromophores whose concentration is determined by the absorbance at 500nm [20].

2.4.2 Reduced glutathione dosage

2,2'-Dithio -5,5'bis (2-nitrobenzoic) acid reacts with the SH groups of glutathione to form a coloured complex which absorbs at 412nm [21].

2.4.3 Catalase assay

Hydrogen peroxide is broken down in the presence of catalase. This destroyed peroxide binds with potassium dichromate to form a green precipitate of unstable perchloric acid, which is then destroyed by heat to form a green complex that exhibits maximum absorption at 570 nm. Catalase activity proportional to the optical density of the complex is obtained from the hydrogen peroxide calibration curve [22].

2.4.4 Histopathological analysis

Histology allows the preparation of tissues/organs for observation under a microscope. The techniques used were those described by Cannet (2004, 2006a, b).

2.4.5 Acute toxicity

The acute toxicity study was carried out in accordance with OECD Directive No. 420 concerning the testing of chemicals [23]. 20 albino rats were used for this study. The ethanolic extract divided into 2 groups, a control group (5 male rats and 5 female rats) 2 test groups (5 male rats and 5 female rats). The rats were fasted for 24 hours. After 24 hours, the test rats received the ethanol extract of *G. senegalensis* leaves at a dose of 2000 mg/kg and the control groups received distilled water. The rats were observed for 24 hours. They were subsequently monitored for 14 days during which their weights were measured and their water and food intake were recorded too. On the 15th day the rats were sacrificed, the blood, kidneys and livers were collected for biochemical and histo-pathological analysis. Table 2 summarizes the distribution and treatment of rats during the acute toxicity study.

2.5 Statistical Analysis

The results obtained were expressed as the mean ± standard deviation. Comparison between groups was performed using the analysis of variance (ANOVA) followed by the post hoc Dunnett test using statistical analysis software Graph Pad Instat version 5.0.

3. RESULTS AND DISCUSSION

3.1 Preventive Effect of Extracts on Hepatic Cytolysis

The Fig. 1 has shown the preventive effect of extracts and the reference drug on hepatic cytolysis induced by paracetamol at a dose of 640 mg/mL. The administration of distilled water to the controlled group, the extracts to the test groups, silymarin to the positive groups followed by the administration of the hepatotoxic agent in this case paracetamol at a dose of 640 mg /kg for 14 days led to an increase in liver serum biochemical parameters.

In the case of ALT, which is a biochemical marker found in high concentration in liver cells and kidney cells, as well as that of AST which is found in cardiac and skeletal muscle cells and liver cells, thus indicating hepatic cytolysis.

A significant decrease in ALT activity is observed with a p-value < 0.001 in the healthy control group, negative control groups and group treated with aqueous maceration extract.

Significant difference with a p-value < 0.01 was also observed in the group of the hydro-ethanolic extract, the decoction extract and a significant decrease with a p-value < 0.05 in the group of infusion extract.

Regarding the ALT parameter, we observed a significant decrease with a p-value < 0.05 and a non-significant difference with a p-value > 0.05 in the other groups.

3.2 Preventive Effect of Extracts on Hepatic Cholestasis

The Fig. 2 shows the preventive effect of plant extracts and the reference drug on hepatic cholestasis induced by paracetamol at a dose of 640 mg/mL. The administration of the extracts and the reference drug leads to a non-significant decrease with a p-value > 0.05 in alkaline phosphatase, albumin and serum total proteins. However, we observe a significant difference with a p-value < 0.05 in comparison with the healthy control group

3.3 Preventive effect of extracts on kidney damage

The Fig. 3 illustrates the preventive effect of extracts and the reference drug on renal damage induced by paracetamol at a dose of 640 mg/mL. The administration of the extracts and the reference drug leads to a non-significant reduction with a p-value > 0.05 of creatinine, urea and uric acid. However, a significant difference is observed with a p-value < 0.05 of the hydro-ethanolic extract in comparison with the negative control group.

Table 2. Distribution and treatment of rats during the acute toxicity study

Groups	Number of rats	Treatment
Groupe 1	5 males rats	
Groupe 2	5 females rats	Receives distilled water (control group)
Groupe 3	5 males rats	
Groupe 4	5 females rats	Receives plant extract at a dose of 2000mg/kg

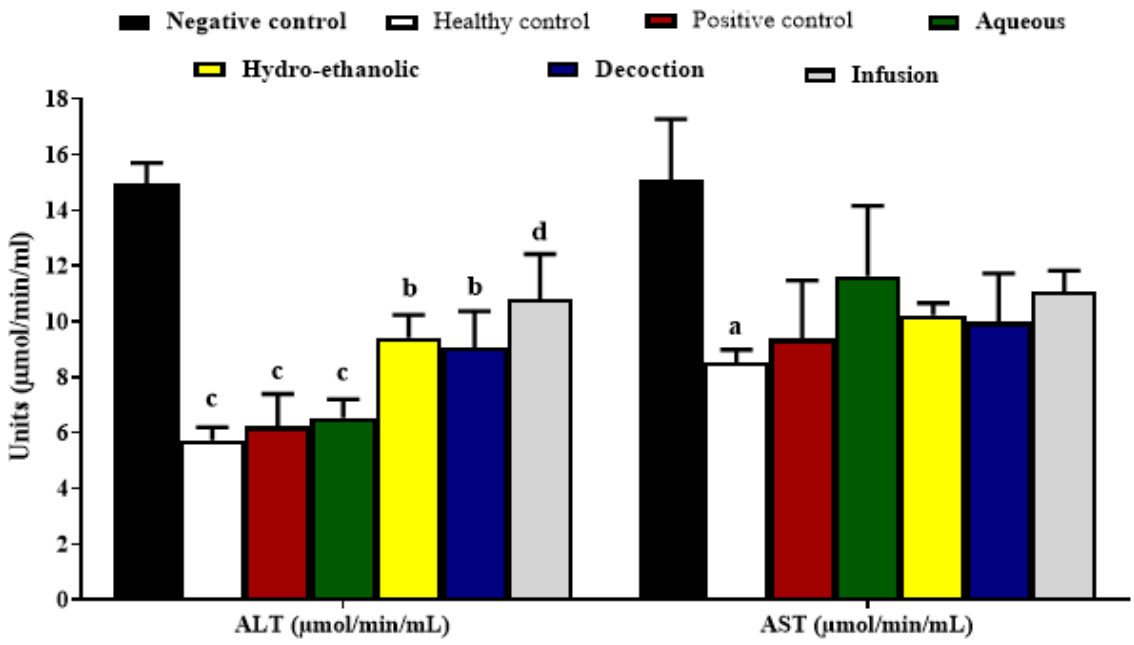

Fig. 4 . Preventive effect of extracts and the reference drug on hepatic cytolysis induced by paracetamol at a dose of 640 mg/mL

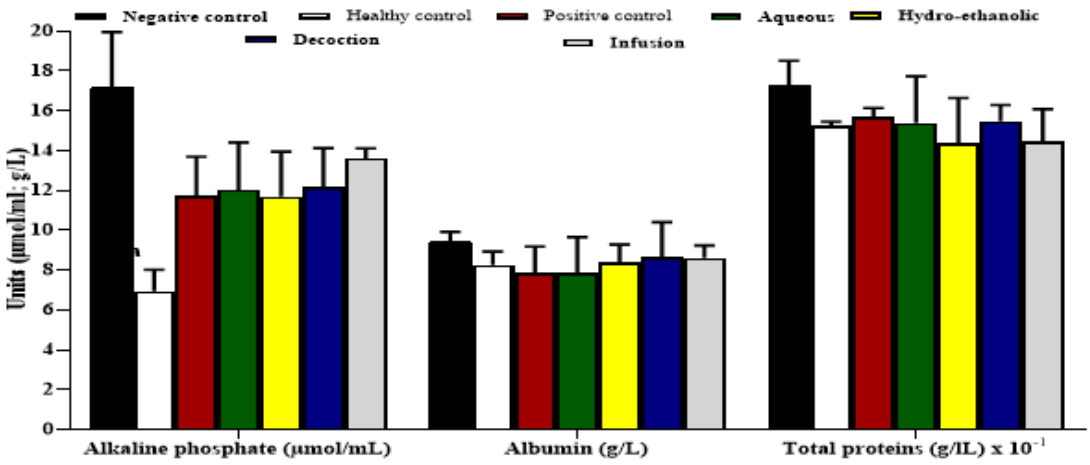

Fig. 5 . Preventive effect of plant extracts and the reference drug on hepatic cholestasis induced by paracetamol at a dose of 640 mg/mL

3.4 Preventive Effect of Extracts on Lipid Profile

The administration of the extracts and the reference drug leads to a significant reduction with a p-value > 0.01 in the triglyceride level in the healthy control group and in the group treated with decoction extract. As shown in Fig. 4. A significant reduction with a p-value < 0.05 in the positive control groups, the infusion extract and a significant difference between the aqueous extract and decoction extract as well as the healthy control group with a p-value < 0.05.

3.5 Preventive Effect of Extracts on in-Vivo Antioxidant Capacity

The pre-treatment of the study groups with the different extracts of the plant, the vehicle and the reference drug, followed by the administration of the hepatotoxic agent in this case paracetamol

led to a reduction in the activity of superoxide dismutase as illustrated in Fig. 5. In the negative control group, significant increase in the healthy group with a p-value < 0.01 and a p-value < 0.05 in the group of the aqueous extract obtained by decoction, a non-significant increase with a p -value > 0.05 in the other study groups.

With regard to catalase and glutathione, a non-significant increase with a p-value > 0.05 of the enzymatic activity is observed in comparison with

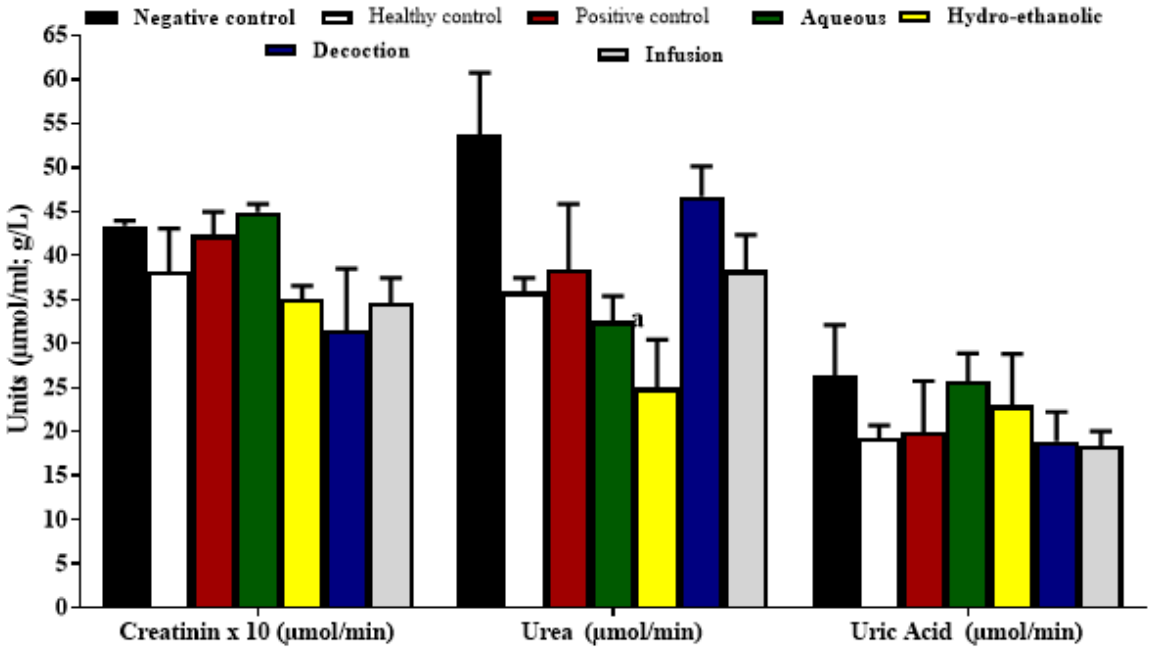

Fig. 6. Preventive effect of extracts and the reference drug on renal damage induced by paracetamol at a dose of 640 mg/mL

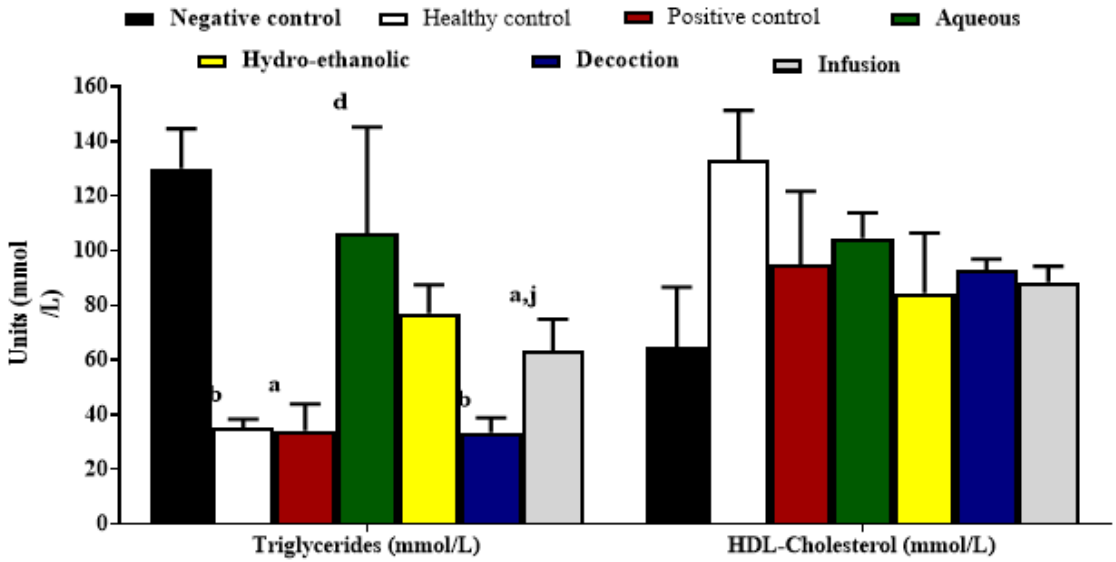

Fig. 7. Effect of extracts and reference drug on lipid profile

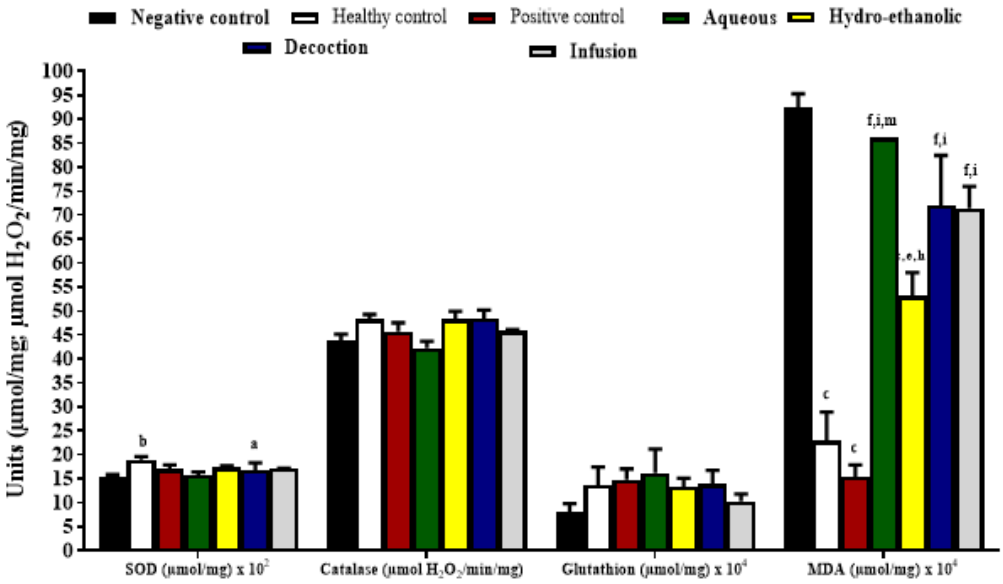

Fig. 8. Effect of extracts and reference drug on in-vivo antioxidant markers

the negative control group. The product of lipid peroxidation symbolized by malondialdehyde was produced in large quantities in the negative control group, a significant inhibition of the production of the product was observed with a p-value < 0.001 in the healthy and positive control groups. The hydro-ethanolic extract showed the greatest inhibitory activity compared to other plant extracts.

The table below summarizes the results obtained during the evaluation of hepatoprotective activity

3.6 Histological Analysis

3.6.1 Histological analysis of the liver

Concerning the liver, the administration of paracetamol leads to obstruction of the centrilobular vein (VC) (Fig. 6). There was also an observation of an aggregation of phagocytic cells indicating inflammation. In the test groups, there was an observed reduction in obstruction of the centrilobular vein, a clear localization of sinusoids (Si) and hepatocytes (He) as illustrated in Fig. 6.

3.6.2 Histological analysis of the kidney

Paracetamol administration did not significantly alter the internal structure of the kidney with the well-identified urinary space (EU), Bowmann's capsule (CB), podocytes (Pd) and well-structured glomerulus (Gl) (Fig. 7).

3.7 Assessment of Acute Toxicity

3.7.1 Evaluation of zootechnical criteria

Weight gain of animals subjected to the toxicity of the hydro-ethanolic extract at a dose of 2000 mg/kg. The administration of the hydro-ethanolic extract to male and female animals in comparison to the groups of animals having received the vehicle in this case distilled water, showed a weight gain of the animals of the test groups with a non-significant difference and a p - value > 0.05. The hydro-ethanolic extract therefore caused weight gain.

$$Weight\ gain\ (\%) = \left(\frac{weight\ of\ the\ animal\ on\ the\ 14th\ day\ -\ Weight\ of\ the\ animal\ on\ the\ 1st\ day}{Weight\ of\ the\ animal\ on\ the\ 1st\ day} \right) x100$$

The Kinetics of the weight evolution of animals subjected to the toxicity of the hydro-ethanolic extract according to OECD line 420 as illustrated in Fig. 9 showed that the kinetics of the weight evolution of the animals in the control groups and the test groups was observed in the evolution of animals from all groups. The plant did not affect the growth of animals.

Table 3. Summary of the results of the biochemical parameters of the effect of plants on hepatotoxicity induced by paracetamol at a dose of 640 mg/kg

Parameters	Negative control	Healthy control	Positive control	Aqueous	Hydro-ethanolic	Decoction	Infusion
ALT (µmol/min/mL)	14.98 ± 1.60	5.73 ± 1.07[c]	6.24 ± 2.32[c]	6.55 ± 1.15[c]	9.43 ± 1.82[b]	9.06 ± 2.63[b]	10.83 ± 3.59[d]
AST (µmol/min/mL)	15.10 ± 4.89	8.55 ± 0.98[a]	9.39 ± 4 17	11.64 ± 4.37	10.23 ± 0.96	9.98 ± 3.51	11.08 ± 1.68
AP (µmole/L)	17.18 ± 6.20	6.94 ± 2.42[a]	11.73 ± 3.92	12.03 ± 4.11	11.70 ± 5.05	1218 ± 3.89	13.62 ± 1.00
Albumin (g/L)	9.45 ± 1.05	8.27 ± 1.50	7.88 ± 2.62	7.87 ± 3.12	8.38 ± 2.03	8.67 ± 3.49	8.62 ± 1.24
Total proteins (g/L)	172.62 ± 28.00	152.46 ± 4.72	157.12 ± 8 44	153.85 ± 40.79	143.69 ± 5073	154.81 ± 16.15	144.81 ± 32.08
Creatinine (µmol/L)	4.34 ± 0.13	3.82 ± 1.10	4.23 ± 0.53	4.49 ± 0.16	3.51 ± 0.33	3.15 ± 1.39	3.46 ± 0.57
Urea (mmol/L)	63.72 ± 37.26	35.85 ± 3.60	38.34 ± 15.03	32.49 ± 4.98	24.96 ± 12.27[a]	54.22 ± 19.31	38.32 ± 8.00
Uric acid (µmole/L)	26.37 ± 11.42	19.32 ± 3.19	19.98 ± 11.49	25 68 ± 5 56	22.98 ± 11.69	18.92 ± 6.65	18.32 ± 2.96
Triglycerides (mmol/L)	130.18 ± 28.88	35.17 ± 7.25[b]	34.15 ± 13.80[a]	106.50 ± 66.90[d]	77.09 ± 23.19	33.54 ± 10.61[b]	63.51 ± 25.66[a,j]
HDL-Cholesterol (mg/dL)	0.65 ± 0.49	1.33 ± 0.41	0.95 ± 0,l 53	1.05 ± 0.16	0.85 ± 0.49	0.93 ± 0.08	0.89 ± 0.12
SOD (µmole/mg proteins)	0.15 ± 0.01	0.19 ± 0.01[b]	0.17 ± 0.02	0.16 ± 0.01*	0.17 ± 0.01	0.18 ± 0.01[a]	0.17 ± 0.002
Catalase (µmol/mg proteins)	43.80 ± 2.73	48.25 ± 2.35	45.77 ± 3.55	42.23 ± 2.58	48.25 ± 3.82	48.36 ± 3.61	45.82 ± 0.54
Glutathione (10^3)	0.80 ± 0.36	1.36 ± 0.85	1.48 ± 0.45	1.62 ± 0.87	1.33 ± 0.39	1.39 ± 0.58	1.03 ± 0.34
MDA	0.92 ± 0.06	0. 20 ± 0.13	0.24 ± 0.17	1.07 ± 0.40	0.63 ± 0.10	0.65 ± 0.21	0.63 ± 0.20
Total proteins	60.58 ± 28.40	96.77 ± 61.02[c]	108.27 ± 31.05[c]	112.05 ± 60.84[f,i,m]	98.62 ± 28.97[c,e,h]	105.19 ± 44.72[f,i]	77.54 ± 26.08[f,i]

Values are expressed in terms of mean ± standard deviation, (n = 5). The comparison between groups is made using the variance test (ANOVA) followed by the Turkey Kramer post hoc test. The difference is significant with a < 0.05; b < 0.01; c < 0.001 for the negative control group; d <0.05; e < 0.01; f < 0.001 for the healthy control group; g < 0.05; h < 0.01; i < 0.001 for the positive control group; j < 0.05; k < 0.01; l < 0.001 for the maceration extract; m < 0.05; n < 0.01; o < 0.001 for the hydro-ethanolic extract; p < 0.05; q < 0.01; r < 0.001 the decoction extract; s < 0.05; t < 0.01; u < 0.001 for infusion extract

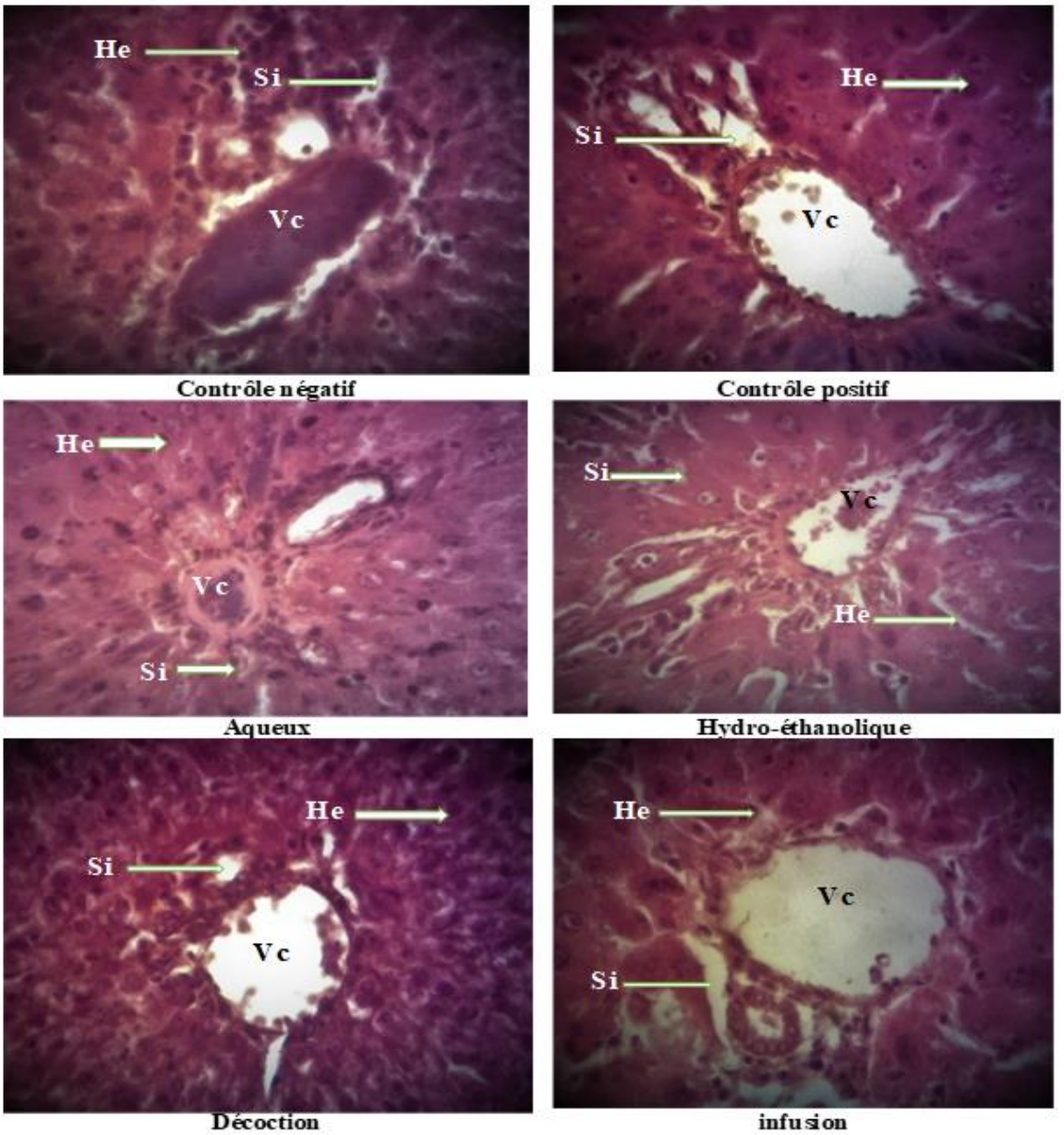

Fig. 9. Histology of the animal's livers from the different analysis groups

3.7.2 Evaluation of the hydro-ethanolic extract on food intake

The administration of the extract to the animals did not significantly affect food intake with a p-value > 0.05 compared to the animals in the control groups (Fig. 10).

3.7.3 Evaluation of the hydro-ethanolic extract on water intake

The administration of the extract to the animals did not significantly affect water intake with a p-value > 0.05 compared to the animals in the control groups (Fig. 11).

The Table 4 presents the elements of the zootechnical criteria for the acute toxicity of the hydro-ethanolic extract, it was observed that the extract did not significantly affect weight gain, food intake and water intake as well as weight change.

3.8 Biochemical Analysis

The administration of the hydro-ethanolic extract led to a non-significant increase with a p-value >

0.05 in the AST of the male test group compared to the male control group and a significant difference with a p-value < 0, 01 of the female test group in comparison with the female control group as seen in table 4. Regarding ALT and total proteins, no significant difference was observed between the groups with a p-value > 0.05.

For creatinine, we observed a non-significant increase with a p-value > 0.05 in the test groups compared to the control groups. The table below summarizes the values of the biochemical parameters obtained after administration of the extract:

The evaluation of liver function involves a serum dosage of biomarkers such as ALT, AST, ALP, GGT, total bilirubin. An elevation of total bilirubin is characteristic of liver damage without specificity while an elevation of ALT is significant of hepatic cytolysis, an elevation of AST could mean liver damage but its presence in other organs such as the heart, the kidneys, and the skeletal muscles make this statement uncertain. An elevation of AP is indicative of cholestasis [24].

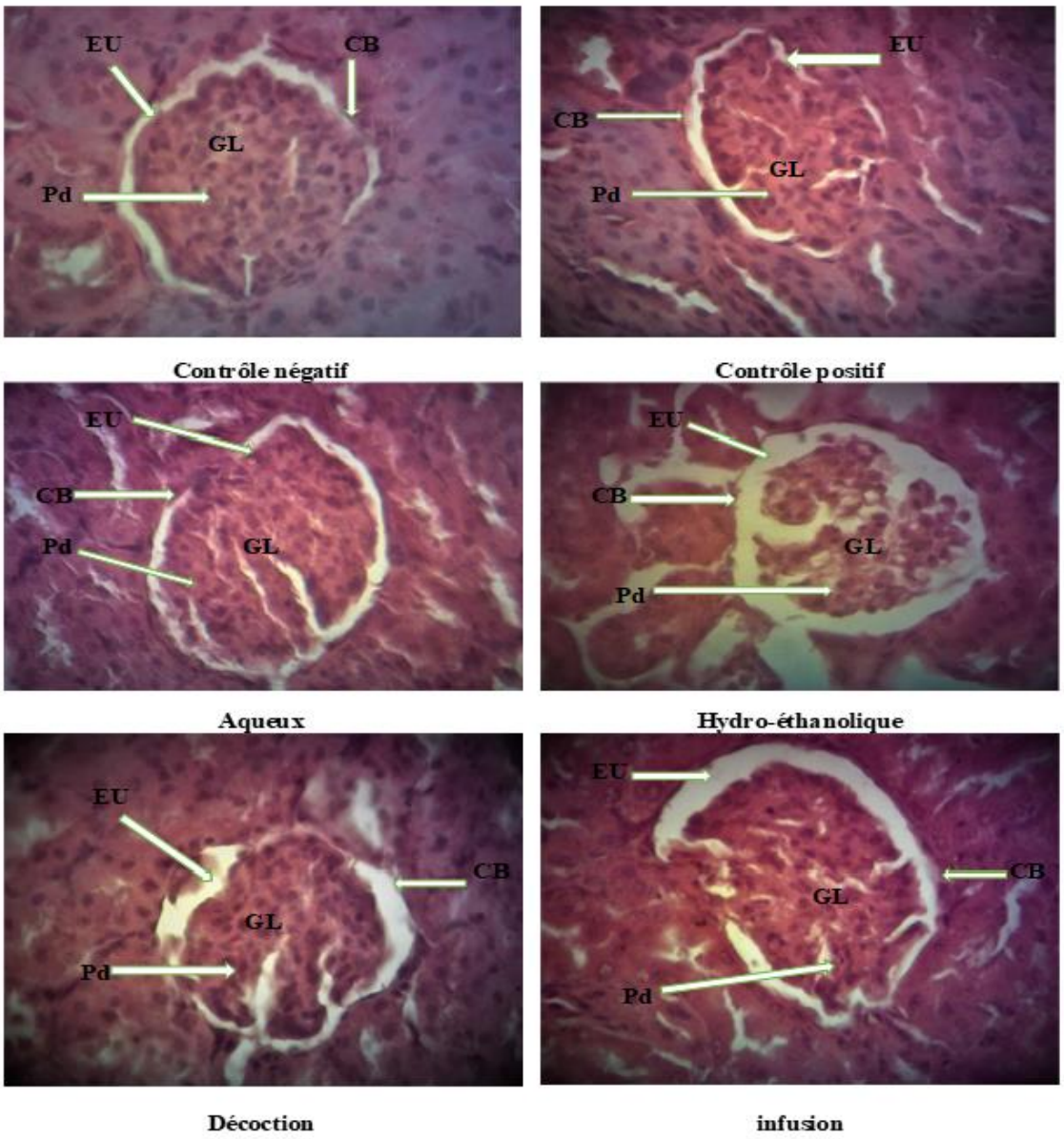

Fig. 10 . Histological section of the kidneys of animals from the different analysis groups

Exploration of the liver function enzymes of rats treated with plant extracts showed that the aqueous extract had a better preventive action on hepatic cytolysis, which led to a significant reduction in the ALT value of 6.55. ± 1.15 µmol/min/ml approaching the value of rats treated with the reference drug.

With regard to hepatic cholestasis, the rats treated with the hydroethanolic extract present a better profile with an AP value of 11.70 ± 5.05 µmol/min/ml, a value close to the value of the rats treated with the drug. Reference. These results corroborate those obtained by Umma L. et *al* in 2023 in their study on the biochemical and toxicological activity of *G. senegalensis* leaf extract [25]. This result can be explained by the presence of molecules acting either through a free radical capture mechanism or by providing endogenous phospholipids necessary for the repair of cells and organelles and restoring the cell membranes of hepatocytes [26]. These results could justify the indication of the aqueous extract of *G. senegalensis* in cases of hepatic cytolysis and the hydroethanolic extract for cases of cholestasis.

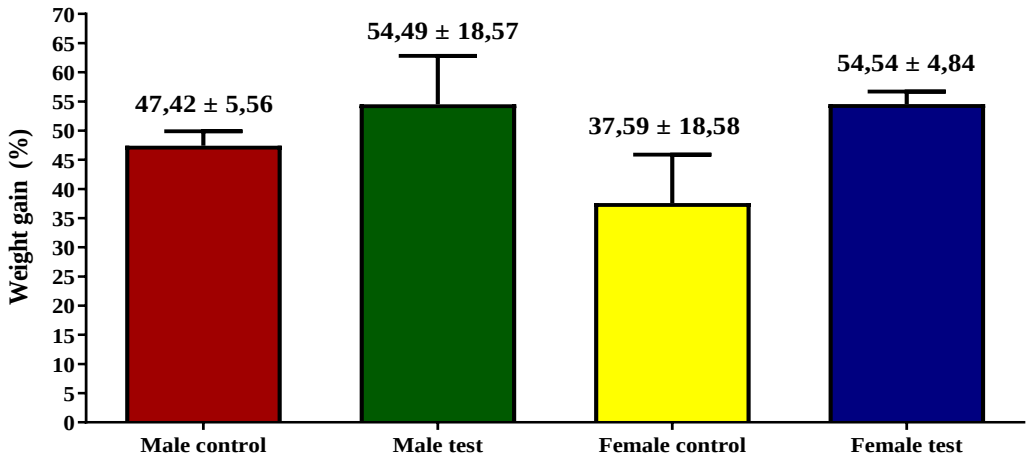

Fig. 11. Evaluation of the acute toxicity of the hydro-ethanolic extract according to OECD guideline 420 on animal weight gain

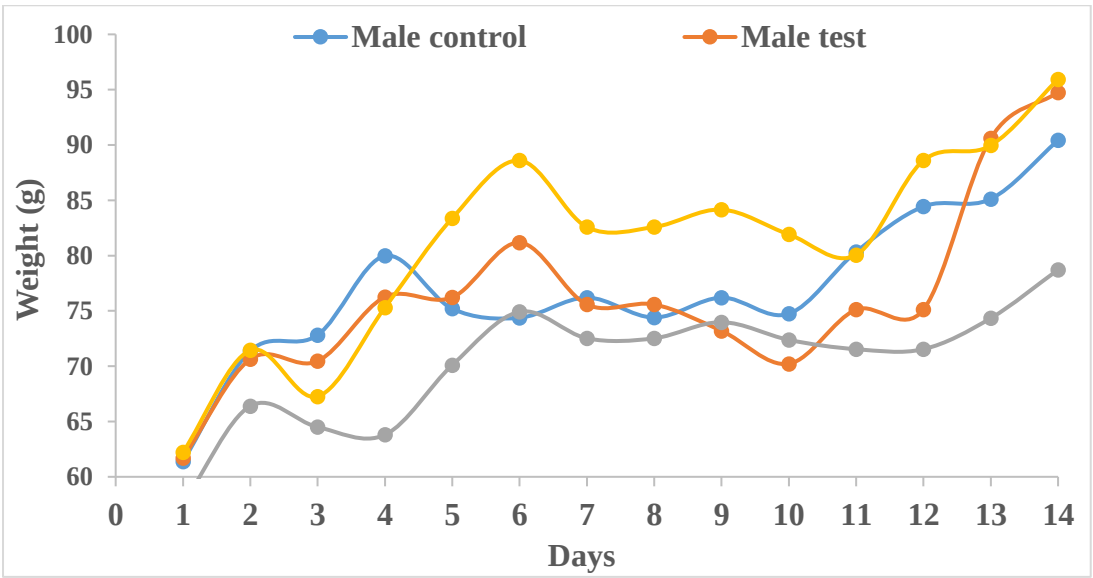

Fig. 12. Kinetics of the weight evolution of animals subjected to the toxicity of the hydro-ethanolic extract according to OECD line 420

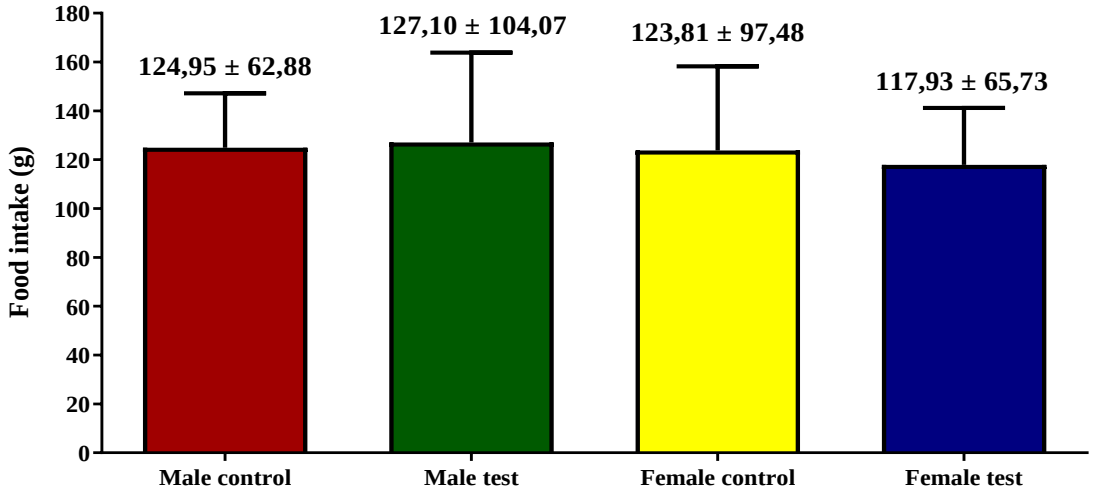

Fig. 13. Evaluation of the acute toxicity of the hydro-ethanolic extract according to OECD guideline 420 on animal food intake

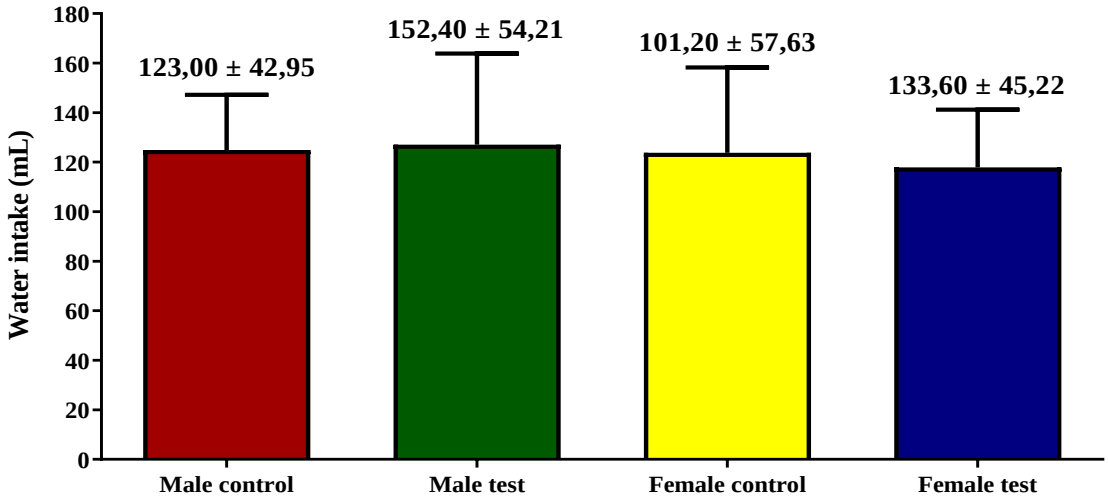

Fig. 14. Evaluation of the acute toxicity of the hydro-ethanolic extract according to OECD guideline 420 on animal water intake

Table 4. Summary of zootechnical criteria for acute toxicity according to OECD guideline 420

Groups	Weight gain (g)	Food intake (g)	Water intake (mL)	Number of death /5
Male control	47.42 ± 5.56	124.95 ± 62.88	123.00 ± 42.95	0/5
Male test	54.49 ± 18.57	127.10 ± 104.07	152.40 ± 54.21	0/5
Female control	37.59 ± 18.58	123.81 ± 97.48	101.20 ± 57.63	0/5
Female test	54.54 ± 4.84	117.93 ± 65.73	133.60 ± 45.22	0/5

Values are expressed in terms of mean ± standard deviation, (n = 3). The comparison between groups is carried out using the variance test (ANOVA) followed by Dunnet's post hoc test

Table 5. Biochemical parameters after administration of plant extracts

Parameters	Male control	Male test	Female control	Female test
AST (µmole/min/mL)	1.96 ± 0.56	4.88 ± 1.68	4.10 ± 0.91	9.08 ± 3.40**
ALT (µmole/min/mL)	4.66 ± 2.41	4.89 ± 1.56	9.69 ± 6.48	9.52 ± 5.15
Total proteins (g/L)	282.77 ± 38.99	329.08 ± 35.32	311.38 ± 43.80	280.77 ± 61.03
Creatinine (µmole/L)	2.40 ± 1.03	2.43 ± 0.69	1.32 ± 1.15	1.82 ± 0.85

Values are expressed in terms of mean ± standard deviation, (n = 5). The comparison between groups is carried out using the variance test (ANOVA) followed by Dunnet's post hoc test

With regard to renal function, a significant reduction in the concentration of urea for the hydroethanolic extract of 24.96 ± 12.27 µmol/L is observed. We also observed a more significant reduction compared to the group treated with the reference drug and a reduction in the creatinine value for rats treated with the extract obtained by decoction of 3.15 ± 1.39 µmol/L more significant than those treated with the reference drug. These results corroborate those obtained by Umma L. et *al* in 2023 [25] in their study on the biochemical and toxicological activity of *G.senegalensis* leaf extract and could be suggestive of a protective nature of renal function.

The exploration of *in-vivo* antioxidant potential is done by determining the concentration of biochemical parameters such as MDA, glutathione, catalase, peroxidase, SOD, ALT, ASAT, LDH, AP [27]. The evaluation of the *in-vivo* antioxidant activity of G. senegalensis leaf extracts showed an increase in the activity of superoxide dismutase in the group of rats treated with the extract obtained by decoction, an increase in the activity catalase and glutathione in the group treated with the aqueous extract. In addition, a reduction in MDA in the group treated with the extract obtained by hydroethanolic maceration 0.63 ± 0.10. MDA is a product resulting from lipid peroxidation responsible for cytotoxicity, mutagenicity and carcinogenicity. It is also responsible for the inhibition of enzymes responsible for cell defence against oxidative stress [28]. These results corroborate those obtained by Abdel wahab et *al* in 2018 in their study on the antioxidant and hepatoprotective activities of *Blepharis linariifolia* PERS and *Guiera senegalensis* j. f. gmel. On hepatotoxicity induced by CCL 4 [29]. These results are justified by the *In vitro* antioxidant capacity by the Folin method, which demonstrates the in-vitro power of *G.senegalensis* leaf extracts, and in particular of its phenolic compounds.

The evaluation of the toxicity of substances is of great importance in the sense that it makes it possible to provide information on the safety of substances, to establish the mechanism of the toxic effect, to explain epidemiological phenomena observed within populations, validated test methods particularly on animals. Toxicity studies can be conducted in acute, subacute and chronic settings [30]. The study of the acute toxicity of the hydroethanolic extract of *G. senegalensis* leaf extracts showed no significant difference in the concentration of biochemical parameters of the groups treated with the aqueous extract and the test groups. These results corroborate those obtained by Umma L. et *al* in 2023 who demonstrated an LD50 of *G. senegalensis* extract greater than 2000 mg/Kg [25]. This could indicate a use with a lower risk of acute toxicity of *G. senegalensis* leaf extract for liver conditions.

Traditional African pharmacopoeia can be defined as the body of knowledge, preparation techniques and use of substances of plant, animal or mineral origin which serve to diagnose, prevent or eliminate an imbalance in physical well-being; mental or social [31]. An improved traditional medicine is a concept that brings together all the medicines designed by a traditional practitioner or a research laboratory, based on knowledge or information from traditional medicine and pharmacopoeia [31]. Africa has a flora extremely rich in its diversity, which represents a great asset for the African pharmacopoeia and contributes to that of the traditional pharmacopoeia. Its exploitation can lead to the development of drugs against diseases present on the continent such as malaria and tuberculosis.

The development of drugs from the African pharmacopoeia would have a significant impact on the economy of the countries of the continent in the sense that the costs incurred for the importation of drugs could be concentrated on the local production of effective, improved traditional drugs [32]. Several varieties of improved traditional medicines exist on the African market. For the purpose of regulation and

control of these different traditional medicines, a classification has been adopted.

The classification of improved traditional medicines was adopted during the first meeting of the WHO Regional Expert Committee on Traditional Medicine held in Harare, Zimbabwe in November 2001 [32]. The development of plant-based medicines is mainly based on ethnopharmacology. Ethnopharmacology is the interdisciplinary scientific exploration of the biological activity of agents traditionally or observed in traditional medicine [34]. The ethnobotanical study begins with: A field study which will have the role of making a census of the practices and elements entering into traditional medicine. A botanical identification, which will allow botanical identification and botanical description of the plant; Laboratory work, which will allow extractions of plant constituents to be carried out, phytochemical study to highlight the groups of compounds present in the plant, pharmacological study to demonstrate the activity of the plant, the toxicity study to determine the safety of the plant;

The formulation of an improved drug for adequate administration to humans; Submission to the regulatory authority with a view to obtaining marketing authorization [35].

4. CONCLUSION

At the end of this study whose main objective was to explore the hepatoprotective activity of extracts of *G. senegalensis* leaves, exploring the biochemical parameters of rats treated with 500mg/kg of leaf extract and 640mg/kg of paracetamol we observed an activity profile depending on the extract. For protective activity in relation to cytolysis, the aqueous extract presented a better profile, for cholestasis, the hydroethanolic extract predominated and for renal protection, the hydroethanolic extract and the extract obtained by decoction presented a better activity profile. Regarding the *in-vitro* antioxidant activity, the hydroethanolic extract demonstrated a better profile, which was corroborated by the in-vivo antioxidant activity.

The study of the acute toxicity of the hydroethanolic extract revealed no alteration at the biochemical and histo-pathological level nor led to the death of the treated animals demonstrating an LD50 lying between 2000 and 5000 mg/Kg. These results demonstrate the strong therapeutic potential of *G.senegalensis* for liver damage, provide justification for its traditional use and could open up perspectives for the development of improved traditional drugs based on its leaf extracts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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