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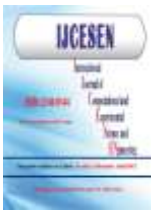

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Research Article

Bioactive profile and safety of *Stipa tenacissima* L medicinal plant: polyphenols, antioxidants, toxicity and anti-inflammatory

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Abstract:

In addition to evaluating the phytochemical content and antioxidant capacity, this study was the first to examine the acute toxicity profile and anti-inflammatory properties of *Stipa tenacissima* L., an endemic plant widely distributed in Algeria known as "Halfa". Although its various and important traditional use, little scientific research has been explored into its critical medicinal properties. The results of these studies show that the aqueous extract contains the most phenolic and flavonoid components and has a higher antioxidant capacity. Anti-inflammatory activity was demonstrated *in vitro* through BSA denaturation inhibition of 89.93% at of 0.5 mg/mL, and *in vivo* use croton oil to produce ear edema in mice, where showed significant inhibition rate of 71.11% at dose of 100 mg/kg. Furthermore, the toxicological evaluation of the two oral doses of the aqueous extract given to mice groups (2 and 5 g/kg) shows that no mortality was seen and the LD_{50} is higher than 5000mg/kg. On histological examination, symptoms of mild toxicity comprised elevated liver enzymes ALT and AST, as well as infiltration of moderate hepatic inflammation, but there was no major renal or cardiac damage. These findings suggest that *S tenacissima* represents an interesting avenue in the development of new natural alternatives to traditional anti-inflammatories.

1. Introduction

Poaceae (Gramineae), also known as the grass family [1]. It's the fourth largest family of plants in the world [2]. It's one of the most important families in the New Flora of Algeria with 116 genera and 284 species, 26 of which are endemic [3]. Many infections and digestive issues have been treated with plants in this family [4]. In traditional medicine, most plants in the Poaceae family are used as antioxidants, anthelmintics, diabetics, astringents, diuretics, antiulcers, and those with anti-inflammatory and anti-hypersensitive properties [5]. *Stipa tenacissima* L. is a Mediterranean perennial herb that grows in North Africa especially in Algeria. It is also called esparto grass; its fiber-rich leaves can reach 1m in length. Esparto grass is frequently used as a possible reinforcement for polymer matrices. Furthermore, because of its unique qualities, it is mostly employed as a noble raw material in the high-quality paper sector. [6].

Given this context, there is an increasing interest in researching the therapeutic potential of less well-studied Poaceae species. Investigating plants like *Stipa tenacissima* L. could offer new insights into their phytochemical composition and reveal new uses in preventive medicine and pharmacology.

Therefore, the purpose of this study was to evaluation this species' toxicological profile and biological activity. Additionally, a phytochemical composition analysis was performed to identify important secondary metabolites that could be in charge of the biological impacts of the plant.

2. Material and Methods

2.1 Plant material

The aerial part of *Stipa tenacissima* L. consists of several branches, surmounted by limbs ranging from 30 to 120 cm in length. samples are collected from the Setif (Wad Al-Sarraf: 17/02/2025) regions and The newly harvested aerial parts meticulously chopped into diminutive fragments to facilitate the extraction of bioactive compound.

2.2 Extraction procedure

Aqueous Extraction (Decoction)

The aqueous (AQ) extraction by decoction was performed following Attah et al [7] with slight modifications based on sample origin. 100g of *S. tenacissima* L. leaves were allowed to infuse overnight after having been boiled for 2h in 500 mL of distilled water. Following filtering, the extract was recovered, dried in an oven, and kept at -4°C.

Ethanolic Extraction (Hot Maceration Using a Reflux System)

The extraction was carried out by maceration utilizing a reflux tool, following the method of Annapandian and Rajagopal [8]. Specifically, 100 g of leaves were soaked in 1200 mL of ethanol and heated under reflux, the mixture was filtered and then concentrated, oven-dried, and also stored at -4°C.

Hydroalcoholic Extraction (Ethanol/Water) – Cold Extraction

The Hydroalcoholic extract of the aerial part of plant was prepared according to Markham with slight modification [9]. Briefly, to extract as many bioactive compounds as possible, 80 g of leaves were soaked in an 80:20 ethanol/water combination for 5days in the ambient temperature. After filtering and concentration, the extract was oven-dried at 45°C, and kept away from light to avoid deterioration.

2.3 Animal material

Albino Swiss mice weighing 16 to 25 g were bought from Algeria's Pasteur Institute. Prior to the experiment, the animals had unrestricted access to

food and water while they were housed in polycarbonate cages for seven days in a laboratory environment (12/12 h light/dark cycle, 23±2°C).

2.4 Phytochemical Evaluation

2.4.1 Qualitative Phytochemical Analysis

Applying the techniques described from the literature, specific assays of phytochemicals were performed, utilizing indicators of color shift, turbidity changes or precipitation reactions, as a means of determining the presence or absence of specific molecules of chemical classes of secondary metabolites namely phenolics, flavonoids, terpenoids, condensed and hydrolysable tannins, quinones, anthraquinones, cardiac glycosides, mucilages, and saponins.

2.4.2 Quantitative Phytochemical Analysis

Total polyphenols content

The total phenolic content was evaluated using the Folin-Ciocalteu test, as described by Li et al. [10]. Three duplicates of each solution are made by combining 100µL of the sample extract with 500µL of Folin-Ciocalteu reagent (diluted 10 times), incubating for 4 min in the dark, and then adding 400µL of $Na_2\ CO_3$ (7.5%). After 1h30min of incubation at room temperature and in the dark, the absorbance at 765nm was measured with a spectrophotometer. The data are shown in milligrams of gallic acid equivalents per gram dry weight (mg GAE/g DW).

Total flavonoids content

Bahorun and his associates used the aluminum trichloride procedure to determine total flavonoid content [11]. Three duplicates of each concentration are prepared by mixing 500µL of extract solution with 500µL of $AlCl_3$ and incubating for 10 min at room temperature and under darkness. The absorbance is measured at 430nm with a spectrophotometer against the blank. The total flavonoid concentration was calculated as quercetin equivalent per gram dry weight (mg QE/g DW).

2.5 Evaluation of *in vitro* antioxidant activity

There are two methods used to evaluate the antioxidant activity of hydroalcoholic, aqueous, and EtOH extracts. In this study we chose DPPH and Ferric Reducing Power (FRAP) tests.

2.5.1 DPPH Radical Scavenging Assay

In accordance with Hanato et al. [12], the plant extracts' antioxidant activity was evaluated. using the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). In the methanol, a DPPH (0.004%) solution was made. Serial dilution was used to create plant extract solutions. To evaluate each dilution, 200 µL of the sample was combined with 800 µL of DPPH in triplicate. The mix was stirred, then incubated at room temperature and in the dark for 30 minutes. The absorbance was then measured at 517 nm using BHT as a reference and a blank made in the same way in methanol.

The following formula (1) was used to determine the percentage of antioxidant activity (I%):

$$I\% = \frac{ABScontrol - ABStreated}{ABScontrol} \times 100 \qquad (1)$$

2.5.2 FRAP Assay: Ferric Reducing Antioxidant Power

The reducing power of the plant extracts was evaluated by a modified method of Chung et al. [13], based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). Which can be monitored spectrophotometrically at 700nm. A variety of dilutions of the plant extract were prepared. 100 µL of the extract solution or BHT, 100 µL of phosphate buffer (0.2M, pH 6.6), and 100 µL of potassium ferricyanide [K3Fe(CN)6, 1%] formed the reaction mixture at each concentration. For 20 min, the tubes were incubated at 50°C in a water bath. Following incubation, 250µL of trichloroacetic acid (TCA, 10%) was added to stop the reaction. Subsequently, the solution was centrifuged at 10 min/3000 rpm. Then 500 µL ferric chloride solution ($FeCl_3$, 0.1%) and 250 µL of distilled water were added to 250 µL supernatant. Absorbance at 700nm with a spectrophotometer against a blank was determined. Effective concentration at 50% (EC50), antioxidant concentration required at an absorbance of 0.5, is how data are expressed.

2.6 Anti-inflammatory activity

2.6.1 Denaturation of Bovine Serum Albumin (BSA)

According to Karthik et al. [14], Different concentrations of aqueous extract were prepared in Tris-HCl (0.02M, pH6.6). Each amount was combined with a 2% bovine albumin aqueous solution in the same proportion. The action mix was first incubated at 37°C for 15 min and subsequently warmed at 72°C for 5 min. Cooling and absorbance were measured at 650 nm. Equation (2) calculated the inhibition percentage of albumin denaturation from extract and Aspirin; Aspirin was used as a positive control. Results were calculated as IC_{50} values.

$$I\% = \frac{A0 - A}{A0} \times 100 \qquad (2)$$

2.6.2 *In Vivo* Anti-Inflammatory Activity

Using an ear-edema model created by topical application of croton oil, Manga et al. [15] examined the anti-inflammatory properties of an aqueous extract from *S. tenacissima* L. Four mice group were given 15µL of an acetone-water solution (1:1) containing 80µg of croton oil as an irritant on the inside surface of the right ear to induce skin irritation. Without croton oil, the left ear received the same volume. The mice were administered orally with extracts at doses of 50 and 100mg/kg after one

hour of croton oil application. The positive control group received 50 mg/kg of indomethacin, while the negative control group received distilled water. Following 6 hours of edema provocation, the ear's thickness was measured utilizing an electronic caliper [16]. The following formula (3) defines the proportion of edema inhibition in comparison to the control group:

$$I\% = \frac{Dcontrol - Dtreated}{Dcontrol} \times 100 \qquad (3)$$

Where *Dcontrol* is the thickness difference in the control group. *Dtreated* Thickness difference for the treated group.

2.7 *In vivo* toxicological study

2.7.1 Acute Toxicity Study

The aqueous extract (AQ) aerial parts of *S. tenacissima* L. were evaluated for acute toxicity using the method outlined in the Organization for Economic Co-operation and Development's guideline [17] (OECD 420, 2001). After a 12-hour fast, 18 female Albino Swiss mice were divided into two treatment groups at random (n = 6 per group) and given oral gavage doses of extract at a rate of 2 g/kg and 5 g/kg, respectively, and control group that was given distilled water. Following oral administration of the extracts, the mice were observed daily for 14 days, as well as for the first 4 and 24 hours, to record clinical symptoms of toxicity and mortality for the reason of determining the lethal dose (LD50). Daily food intake and weekly body weight were monitored.

2.7.2 Hematological and Biochemical Analysis

Following anesthesia and sacrificed by rapid decapitation at the conclusion of the experiment, the animals' blood was collected for examination in heparin and hematocrit (EDTA) tubes.

Hematological studies were performed using blood obtained in EDTA tubes: RBC: red blood cells, WBC: white blood cells, HTC: hematocrit, PLT: platelets, HGB: hemoglobin, VGM: mean corpuscular volume, MCH: mean hemoglobin concentration, MCHC: mean corpuscular hemoglobin concentration, MPV: mean platelet volume.

Heparin-tubed blood was centrifuged for 10 minutes at 5000 rpm, recovered, and kept at -4°C until it was time for biochemical analysis. For liver tests (ALAT: alanine aminotransferase, ASAT: aspartate aminotransferase, PAL: alkaline phosphatase), renal tests (UREE and CREA: creatinine).

2.7.3 Histopathological Analysis

The removed liver, kidneys, spleen, heart, stomach and brain are fixed in 10% formalin for one week. The specific organs liver, kidneys, and heart were then, the following steps embedding, sectioning and staining. The samples are dehydrated by passing through three successive baths of ethanol, then cleared in two 20min baths of toluene and embedded in paraffin (the operation is automated using a SLEE MTP machine). The final embedding is then carried out in metal molds (MEDITE embedding centre TES 99). The obtained paraffin blocks are then cut by microtome (LEICA). The 5µm thick sections are spread on slides and dried for one hour at 37°C, rehydrated, stained with hematoxylin-eosin (MEDITE linear strainer COT 20). For histological evaluation, the produced slides were examined under a light microscope.

Statistical analyses

The Mean ± SD represents the results of the *in vitro* study. The linear regression approach uses the curve % inhibition = f(concentrations) to determine the IC_{50} values. The Graph Pad Software Prism (Version 5.01) is used to create the test graphs. The mean± standard error of the mean (SEM) was used to display the results of the *in vivo* tests. Following the univariate ANOVA, the Dunnet/Tukey test for multiple comparisons and the calculation of significant rates are used to evaluate the difference between the control and the various groups. *P*-values ≤0.05 are generally considered as statistically significant.

3. Results and Discussions

3.1 Phytochemical Evaluation

3.1.1 Qualitative Phytochemical Analysis

The phytochemical analysis of *S. tenacissima* L. extracts indicated the presence of several major classes of secondary metabolites, including polyphenols, flavonoids, quinones, tannins, terpenoids, and saponins; however, anthraquinones, cardiac glycosides, and mucilage were absent (Table1).

Table 1. *Phytochemical Screening tests of extracts of Algerian S. tenacissima L aerial parts*

Secondary metabolites	AQ Extract	EtOH	EtOH-H_2O
Phenols	+++	+++	+++
Flavonoids	+++	++	++
Terpenoides	+	++	+
Tannins	++	++	++
Quinones	+++	+++	+++
Anthraquinones	-	-	-
Glucosides cardiac	-	-	-
Mucilages	-	-	-
Saponins	+	+	+

+++: strongly present, ++: moderately present, +: weakly present, -: absent

It's approved by El Bouchti et al. 2021, who reported the presence of phenolic compounds of the same species from the Moroccan area [6]. This qualitative screening confirms the presence of compounds known for their biological activities and potential health [18].

3.1.2 Quantitative Phytochemical Analysis

Determination of total polyphenols content

The results clearly show that the aqueous extract contains the highest amount of phenolic compounds with 97.02±0.56 mg GAE/g, followed by the ethanol extract with 73.71±0.17mg GAE/g however the hydroalcoholic extract 49.79±0.99mg GAE/g (Table 2).

Determination of total Flavonoids content

According to the findings, the aqueous extract contains the highest amount of flavonoids content 50.45±1.65mg EQ/g, followed by ethanol extract 38.65±0.68 mg EQ/g and then the hydro-alcoholic extract which has the lowest flavonoid content 15.41±0.77mg EQ/g (Table 2).

Table 2. *Total polyphenol and flavonoids contents of EtOH, AQ and EtOH-H_2O extracts of S. tenacissima. L*

Extract	Total phenolic content mg GAE /g	Total flavonoid content mg EQ/g
AQ	97.02±0.56	50.45±1.65
EtOH	73.71±0.17	38.65±0.68
EtOH-H_2O	49.79±0.99	15.41±0.77

Out of the three generated extracts, the aqueous extract contained the greatest overall levels of polyphenols and flavonoids, according to the quantitative evaluation of these compounds. The extraction process is largely responsible for the difference between our results and the data from the literature, as different solvent types with different polarities affect the extraction efficiency of phenolic compounds. El Bouchti et al. (2021) used methanolic fractions, which involve separation techniques that isolate phenolics components, naturally increasing the total phenolic content values [6].

3.2 *In vitro* antioxidant activity

3.2.1 Determination of the antiradical activity of extracts by DPPH Assay

The results of antioxidant activity showed a clear difference in among the three extracts, as indicated by the IC_{50} values. With an IC_{50} concentration of 0.062±0.001mg/mL, the plant's AQ extract exhibited the highest activity, followed by the ethanol extract at 0.077±0.003mg/mL. However, the lowest activity was observed for the hydro-alcoholic extract with 0.088±0.001mg/mL compared with BHT IC_{50}=0.026± 0.001mg/mL as shown in (Fig. 1) and these findings are consistent with many previous research on the antioxidant effects of plant extracts.

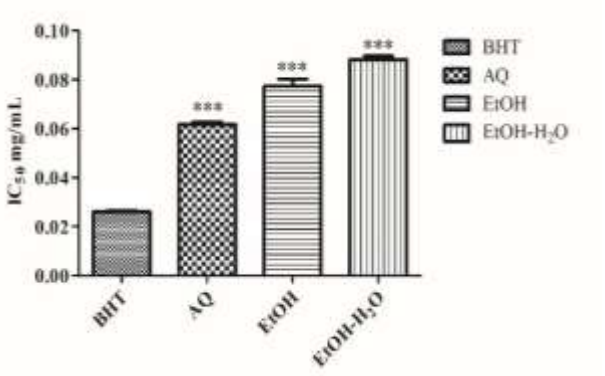

Figure 1. *A comparison between the concentration of EtOH, Aqueous and Hydroalcoholic extracts of S. tenacissima L. and BHT inhibited 50% of the DPPH radical (n=3).*

3.2.2 Ferric Reducing Antioxydant Power test (FRAP)

The reducing power of the three extracts varied significantly, with EC_{50} values of 0.262±0.007mg/mL, 0.266±0.004mg/mL, and 0.335±0.006mg/mL for hydroalcoholic, aqueous, and EtOH extracts, respectively, when compared to BHT as a positive control (EC50 = 1.089 ± 0.016 mg/mL) as shown in Fig. 2.

These result demonstrate that Esparto grass's aqueous and hydro-alcoholic extracts continue to exhibit remarkable natural antioxidant potential.

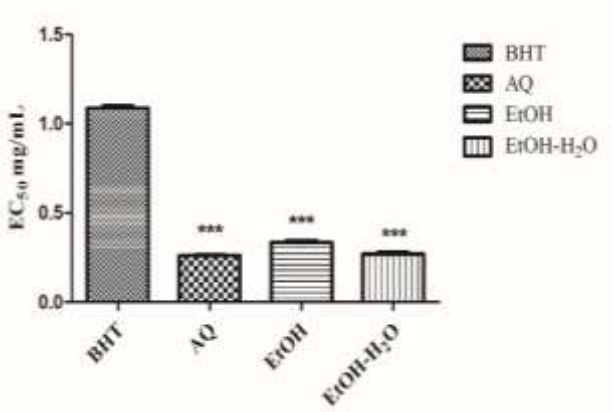

Figure 2. *A Comparison of the reducing power of EtOH, Aqueous and Hydro-alcoholic extracts of S. tenacissima L. Data were presented as mean EC_{50} ± SD (n = 3) compared to BHT standard*

The aqueous extract has the greatest level of antioxidant activity, as shown by better results at DPPH and FRAP doses than the other extracts (ethanolic and hydroalcoholic). This superiority is explained by its high content of flavonoids and polyphenols, both of which have antioxidant capacity, as reported by Irina and Mohame (2012) and Stagos (2020) [19-20]. Previous research has also validated this antioxidant activity in the Poaceae family, Chitindingu et al. (2007) [21] found substantial antioxidant activity in *Brachiaria brasiliensis* and *Panicum maximum*, and Pushparaj and Urooj (2014) [22] confirmed this property in

Pennisetum typhoideum. Furthermore, El Bouchti et al. (2021) found antioxidant activities in *Stipa tenacissima* L., which confirms the current study's findings [6].

In comparison to ethanolic and hydroalcoholic extracts, the AQ extract of *S. tenacissima* L. was found to have a strong reducing capacity and radical scavenging activity against DPPH. These findings are well correlated with phenolic content, indicating that it was able to extract a significant amount of flavonoids and polyphenols, which are responsible for antioxidant activities in many plant species.

To choose a safe dosage and evaluate its pharmacological usefulness, toxicological and anti-inflammatory investigations of *S. tenacissima* L AQ extract are required for the first time to confirm its safety and assess its toxicological profile.

3.3 Anti-inflammatory activity determination

3.3.1 BSA denaturation test

The inhibitory effect of the AQ extract on denaturing BSA protein was compared with standard aspirin at various concentrations (5, 2.5, 1.25, 0.625 mg/mL), as shown in Fig.3.

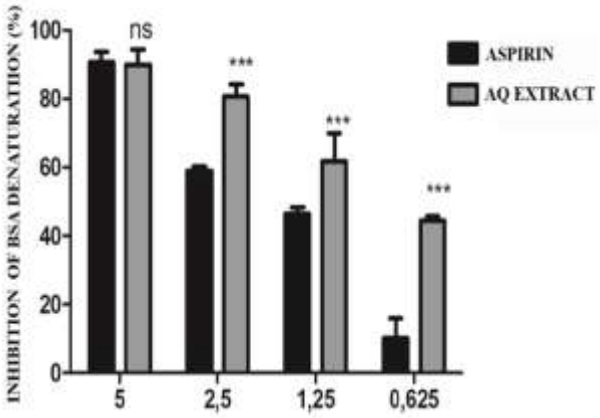

Figure 3. *Values of Aqueous extract of S. tenacissima L compared to aspirin standard inhibitory effect at various doses (Mean±SD, n=3) (***p < 0.001)*

The results showed that at a concentration of 5mg/mL, the extract exhibited high effects with an inhibition rate of 89.93%. which was extremely near to that of aspirin (90.69%), with no significant difference, indicating a comparable impact at this dose. At lower dosages (2.5, 1.25, and 0.625 mg/mL), the extract outperformed aspirin, with inhibition rates of 82.77% vs. 58.94%, 66.03% vs. 46.48%, and 44.42% vs. 10.18%, respectively, with highly significant differences. These findings indicate that *S. tenacissima* exhibits significant anti-inflammatory benefits even at low concentrations, indicating its value as a natural producer of substances that reduce inflammation, particularly in situations requiring plant-based alternatives or reduced side effects [23].

3.3.2 Croton oil induced ear edema

The AQ *S. tenacissima* extract showed significant beneficial effects on inflammation in a model of ear edema produced by croton oil (fig.4). Furthermore, treatment at 50 mg/kg resulted in a 53% inhibition of edema, which, in contrast to the control, was much higher. With an inhibition of about 71%, the 100 mg/kg dose had a good potential for therapeutic use and wasn't significantly different to the reference treatment indomethacin (78%).

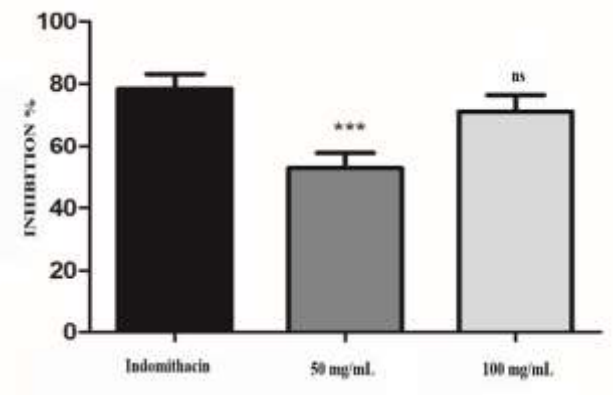

Figure 4. *Aqueous extract anti-inflammatory activity on mice ear edema induced by croton oil (Mean ±SEM, n=6) (***p < 0.001)*

These results indicate to an anti-inflammatory effect that is dose-dependent, which is most likely mediated by modulation of key inflammatory pathways such as protein kinase C inhibition or xanthine oxidase activity, which are both known to contribute to the growth of reactive oxygen species (Rahman et al., 2011, Whitehouse et al., 1998). Other plant extracts, such as *Cnidoscolus aconitifolius*, have shown similar benefits, mainly by downregulating pro-inflammatory cytokines and inhibiting oxidative stress [24]. Overall, the AQ extract of *S. tenacissima* L appears to have anti-inflammatory properties by interfering with both enzymatic and cellular components of the inflammatory response.

3.4 Toxicological study (*In vivo*)

Observation of behavior and table of animal clinic

In the 4h after the extract was administered, the acute toxicity test of the *S. tenacissima* L. AQ extract at the various tested doses (2000 and 5000 mg/kg) revealed no toxicity or mortality, but some obvious behavioral changes were observed, including agitation with some trembling, piloerection, and tachycardia. It showed an overeating act on the first day, especially in the group treated with 5000 mg/kg. However, slight diarrhea and significant cyanosis were observed in the female mice treated on day 7, especially in the mice of the group treated with the highest dose table 3.

Table 3. *Clinical chart for observing mice behavior of the AQ extract of S. tenacissima. L 14 days of treatment*

Parameters	Groups		
	Dose 1	Dose 2	Control
Stimulation			
Agitation	+	+	-
Convulsion	-	-	-
Vocal Fremitus	-	-	-
Eye/skin irritation	-	-	-
Stereotype movement	-	-	-
Touch-response	-	-	-
Salivation	-	-	-
Tachycardia	+	+	-
Trembling	+	+	-
Inhibition			
Abdominal constractions	-	-	-
Extention	-	-	-
Ptosis	-	-	-
Sleep longer	-	-	-
Release of the part	-	-	-
Other			
Cyanosis	+	++	-
Apetite (increased)	++	+++	-
Diarrhea	-	-	-
Whimpering	-	-	-
Urination	-	-	-
Piloerection	+	+	-
Number of deaths	0	0	0

-No effect, + slight effect, ++ medium effect, +++ major effect

Consequently, these findings show that a single dosage had no negative effects or mortality, indicating that the average LD_{50} for female mice was more than 5000 mg/kg. Therefore, according to the acute toxicity classification approach [25], this extract could be classified non-toxic. These findings are harmonious with those of Abidoye et al. (2017), who found no mortality after administering an extract of *Oryza barthii*, a species of plant in the same family, however the clinical signs differed [26].

Body weight changes and food consumption

The results demonstrated that the female mice's body weight is not affected after treatment with AQ extract at different doses (fig.5A). The effect on the growth response could be the result of an increase in food intake (this is well noticed on the 1st day after treatment) (fig.5B).

To compare our results with the literature, we did not find any work on the toxicity of the aqueous extract of *S tenacissima* L. However, our findings agree with those of [27–28], who demonstrate that there were no appreciable changes in the average body weight of rats over the course of the 14-day test using plant extract from the Poaceae family. These results correlate with the reality that female mice consumed much more food than the others in the control group, particularly when receiving a dose of 5 g/kg.

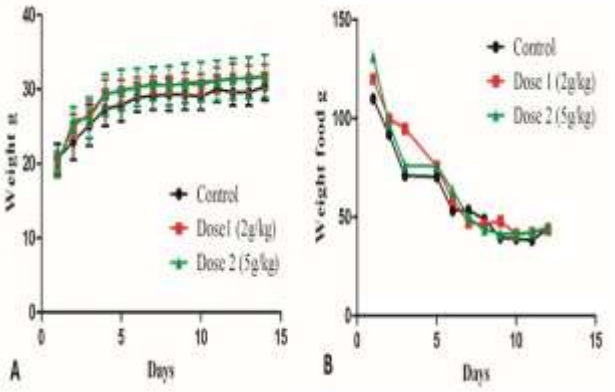

Figure 5. *Changes in body weight (**A**) and food consumption (**B**) of female mice treated with AQ extract, compared to the control group, during 14 days. Mean ± SEM (n=6) is used to represent values.*

Organs weight

According to Table 4, The liver, kidneys, spleen, heart, stomach, and brain in various doses were all examined, and the relative weight of the organs revealed no significant variations.

Table 4. *Female mice's relative organ weights after a 14-day of treatment with an AQ extract of the aerial part of S. tenacissima L.*

Organs	Control	Dose 1 (2g/Kg)	Dose 2 (5g/Kg)
Liver	5.514±0.390	5.390±0.269	5.442±0.203
Kidneys	1.257±0.126	1.216±0.160	1.044±0.075
Spleen	0.379±0.076	0.309±0.051	0.296±0.082
Heart	0.429±0.054	0.470±0.065	0.450±0.066
Stomach	1.097±0.114	1.263±0.240	0.989±0.238
Brain	1.382±0.406	1.307±0.198	1.376±0.175

Mean ± SEM is used to express values (n=6).

Haematological parameters

The study of haematology carried out after 14days of administration of aqueous extract of *S. tenacissima* L.(Table 5) revealed a slight increase in white blood cells (WBC) observed This suggests inflammatory, infectious, or tissue damage, probably caused by the high concentration of extract supplied [29], the proportion of lymphocytes showed a clear increase for both doses, this increase may boost immune function through immunostimulating activities, Based on Jorum et al. (2016)'s study on the effects of Carissa edulis leaf extract [30], the platelet level was significantly higher than in the control group, most likely as a result of a secondary (indirect) effect of general hemopoietic stimulation linked to hemolysis, hemorrhage, or inflammation [31].

Thus, these results suggest that the AQ extract influences the haematopoietic system by modulating blood cells, which could have implications for the immune response and inflammatory processes. The study by Bidié et al. (2016), which explored the effects of *Chrysophyllum perpulchrum* (Mildbr. ex Hutch. & Dalziel) total AQ extract on haematological and biochemical parameters, is consistent with our results. Their results showed that the extract significantly increased white blood cells and platelet counts, suggesting potential immunostimulatory and antioxidant properties of the plant [32].

Table 5*. Hematological parameter changes in female mice following a 14-day of treatment with AQ extract of S. tenacissima L aerial parts*

Parameters	Control	Dose 1	Dose 2
WBC×10^9/L	5.87±0.21	**7.07±1.36*****	**11.03±2.3*****
LYMPH#×10^9/L	4.67±1.11	4.23±0.21^{ns}	**9.27±2.57****
MID#×10^9/L	1.50±0.36	1.53±0.15^{ns}	1.27±1.02^{ns}
GRAN# ×10^9/L	0.90±0.30	1.1±0.17^{ns}	1.17±0.70^{ns}
LYMPH%	65.37±3.16	**72.27±3.11*****	**72.2±4.8*****
MID %	21.53±4.47	18.27±0.50***	18.4±5.62***
GRAN %	13.10±4.83	14.8±3.73^{ns}	14.4±3.39^{ns}
RBC×10^12/L	8.23±0.64	7.97±0.40^{ns}	7.87±0.16^{ns}
HGB g/dL	14.63±1.07	15.13±0.64^{ns}	14.57±0.32^{ns}
HCT %	42.00±3.20	42.97±1.46^{ns}	40.9±0.17^{ns}
MCV fL	51.03±1.33	53.93±0.84^{ns}	51.97±1.19^{ns}
MCH pg	17.83±0.64	18.5±0.26^{ns}	18±0.35^{ns}
MCHC g/L	349±4.00	352±5.19^{ns}	356.3±8.96^{ns}
RDW-CV	0.18±0.02	0.19±0.03^{ns}	0.19±0.02^{ns}
RDW-SD fL	31.47±3.57	36.3±5.89^{ns}	34±4.27^{ns}
PLT×10^9/L	187.3±42.4	**501±18.08*****	**684±12.5*****
MPV fL	6.2±0.82	6.13±0.29^{ns}	6.13±0.40^{ns}
PDW	15.57±0.70	15.13±0.06^{ns}	15.33±0.35^{ns}
PCT mL/L	1.14±0.13	2.05±0.22^{ns}	1.86±1.35^{ns}
P-LCC×10^9/L	15.33±9.24	48.67±8.96^{ns}	25.67±0.58^{ns}
P-LCR	0.075±0.08	0.064±0.01^{ns}	0.069±0.02^{ns}

*Mean ± SEM (n=6) is used to express values compared to the control group (ns: not significant, *P<0.05, **P<0.01, ***P<0.001). (**ns**: not significant, *P<0.05, **P<0.01, ***P<0.001) compared with the control group. **WBC**: White Blood Cells, **LYMPH**: Lymphocyte Count, **MID** :Mid-size Cells Count (monocytes, eosinophils, basophils), **GRAN**: Granulocyte Count, **LYMPH** % : Lymphocyte Percentage, **MID** % : Mid-size Cells Percentage, **GRAN** %: Granulocyte Percentage, **RBC**: Red Blood Cells, **HGB**: Hemoglobin, **HCT** : Hematocrit, **MCV** fL : Mean Corpuscular Volume (femtoliters), **MCH** pg : Mean Corpuscular Hemoglobin (picograms), **MCHC**: Mean Corpuscular Hemoglobin Concentration, **RDW-CV** : Red Cell Distribution Width-Coefficient of Variation, **RDW-SD** fL : Red Cell Distribution Width-Standard Deviation (femtoliters), **PLT** : Platelets, **MPV** fL : Mean Platelet Volume (femtoliters), **PDW** : Platelet Distribution Width, **PCT**: Plateletcrit, **P-LCC**: Platelet Large Cell Count, **P-LCR** : Platelet Large Cell Ratio (%)*

Biochemical parameters

Compounds of AQ extract of *S. tenacissima* L. aerial parts show signs of hepatotoxicity, indicated by elevated AST and ALT. While, the renal function remains relatively preserved, with only slight urea elevation (not significant) (table 6).

Table 6. *Differences in biochemical parameters in female mice after a 14 Days treatment with AQ extract of S. tenacissima L.*

Parameters	Control	Dose 1	Dose 2
Urea g/L	0.18±0.03	0.19±0.13^{ns}	0.26±0.02^{ns}
Creatinine mg/L	6.61±0.13	6.84±0.21^{ns}	6.84±0.21^{ns}
AST UI	28.27±0.85	**41.37±1.40*****	**42.37±1.02*****
ALT UI	27.92±0.11	**39.01±1.04*****	**40.01±1.14*****
ALP UI	430.5±21.92	**264±6.55*****	**164±4.55*****

*Mean ± SEM (n=6) is used to express values compared to the control group (ns: not significant, *P<0.05, **P<0.01, ***P<0.001). Aspartate Aminotransferase (**AST**), Alanine Aminotransferase (**ALT**), and Alkaline Phosphatase (**ALP**).*

These results are similar to those of a study on *Bambusa vulgaris* (Poaceae) by Abe et al. (2020), where an increase in the hepatic enzymes AST and ALT was also noted after an aqueous extract was administered, but the renal parameters stayed largely unchanged [33].

Histopathological Examination

Hepatic Histology

Microscopic histological analysis of the livers of mice treated under acute toxicity conditions revealed significant differences between the doses studied and control group.

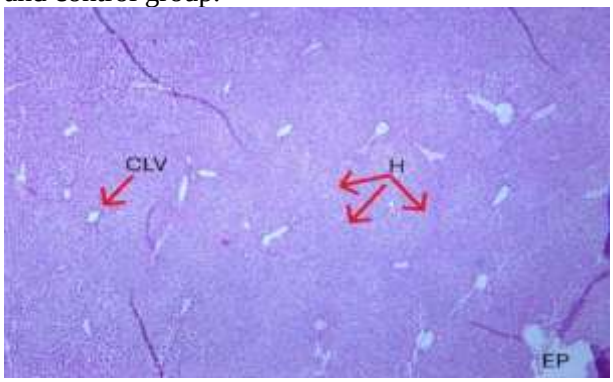

Figure 6. *Hepatic histological cuts of control group of mice (hematoxyline / eosin, x4). H: Hepatic cells, CLV: Centrolobular vein, EP: Espace porte.*

A histological examination of the liver of mice treated dose 1 presenting vascular congestion (Fig. 7.A) reveals a marked dilatation of the sinusoids, indicating intrahepatic blood accumulation. This congestion is typically followed by cellular ballooning of the hepatocytes (Fig. 7.B), which is characterized by cytoplasmic enlargement caused by an alteration in membrane permeability and a buildup of intracellular water, indicating reversible cell stress (light color compared to the control).

Furthermore, bi-nucleated cells are detected (Fig. 7.C), indicating liver regeneration activity, as hepatocytes begin the proliferation phase to restore liver mass following a lesion (Fig. 7).

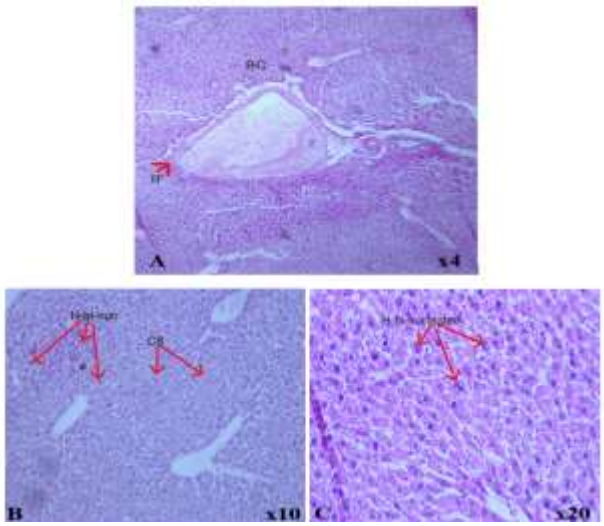

Figure 7. *Hepatic histological sections of treated group of mice with 2 g/kg of S. tenacissima L. AQ extract (hematoxyline / eosin).* ***A****: Vascular congestion x4,* ***B****: Cellular ballooning+ Hepatic regeneration,* ***C****: Hepatic regeneration activity. BC: Blood congestion, CB: Cellular ballooning, IF: Inflammatory infiltrate, H-bi-nucleated: hepatocytes bi-nucleated,*

Dose 2 Compared to dose 1, the hepatocytes exhibit a notable cellular ballooning (Fig. 8.A), appearing larger with a clear and vacuolated cytoplasm, along with vascular congestion in the microscopic histological aspect of the liver. Furthermore, an increase in bi-nucleated cells a classic indicator of hepatic regeneration activity is commonly seen (Fig. 8.B). A local inflammatory response (Fig. 8.C) is indicated by the moderate lymphocyte infiltration that is frequently linked to this regeneration. These lymphocytes, which are normally located in the sinusoids and portal spaces, function in immune control and tissue repair regulation (Fig. 8).

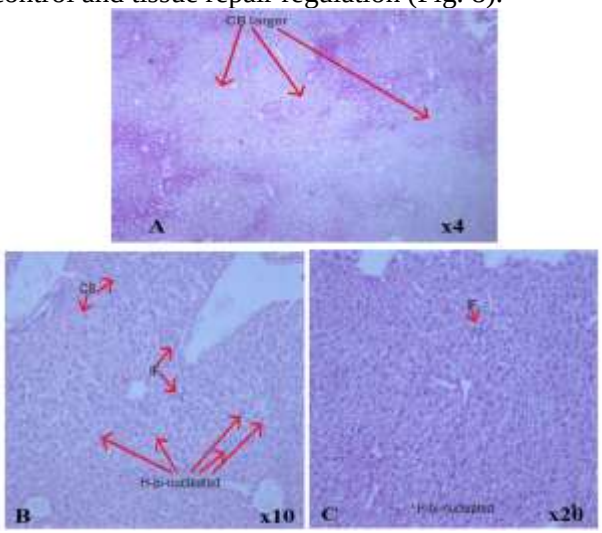

Figure 8. *Hepatic histological cuts of treated group of mice with 5 g/kg of S. tenacissima L. AQ extract (hematoxyline / eosin).* ***A****: Large cellular ballooning (x4),* ***B****(x10) and* ***C****(x20): Cellular ballooning+ Inflammatory response + Hepatic regeneration. CB: Cellular ballooning, IF: Inflammatory infiltrate, H-bi-nucleated: Hepatocytes bi-nucleated.*

Vascular Congestion and Cellular Bloating resulted in sinusoid dilation and substantial blood engorgement. This congestion indicates a disturbance in hepatic blood flow, which could be caused by a toxic effect on blood flow or a change in systemic cardiac/vascular function. Parallel cellular ballooning of hepatocytes is a sign of acute cellular stress caused by decreased membrane permeability and water buildup.

The presence of bi-nucleated cells, which frequently increases with dose 2, indicates liver regeneration. Their presence indicates the liver's attempt to repair the damage and recover its functional mass following toxic aggression. The increase in this occurrence with the higher treatment (dose 2) indicates that the caused lesion is larger, necessitating a more aggressive regeneration response.

The extensive evaluation of dose 2 specifies a "moderate infiltration of lymphocytes, indicating a local inflammatory response". This lymphocytic presence indicates that the immune system is activated in response to damage to tissues, assisting with monitoring and repair. The absence of an explicit description of a lymphocytic infiltration in the analysis of dose 1 could indicate a less prominent inflammatory response or a different type of infiltrate.

While, the differences between the two doses indicate a dose-response connection in terms of lesion severity and liver response intensity.

These results suggest that in situations of aggressiveness, the liver activates its defence and repair mechanisms, but the degree of the reaction is directly proportional to the dose administered indicating a dose-dependent risky potential.

Renal Histology

Histological sections of the kidney of control group (Fig. 9A) and groups treated (Fig. 9B) and (Fig. 9C), stained with hematoxylin and eosin, showed no morphological abnormalities, toxicity or tissue damage. This indicates that the plant has no adverse effect on renal morphology, even at high doses, with the treated animals possessing the same renal architecture as the controls.

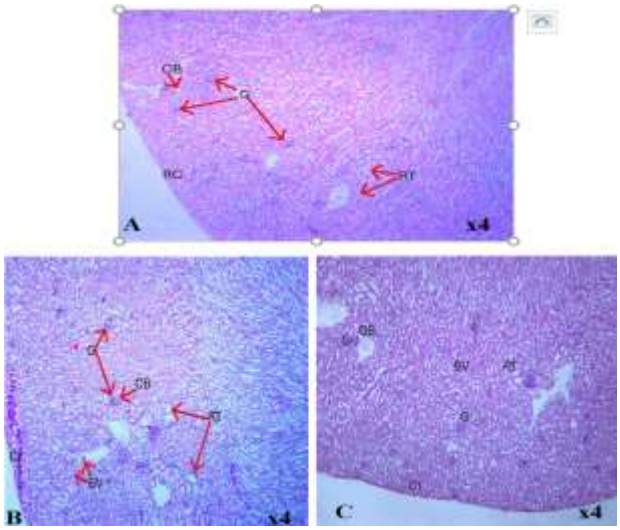

Figure 9. *Renal histological cuts of control group and treated mice with S. tenacissima L. AQ extract. (hematoxyline & eosin, x4).* ***A****: control group,* ***B****: treated group with 2 g/kg,* ***C****: treated group with 5 g/kg. Cr: renal capsule, RT: renal tube, CB: Bowman capsule, G: Glomerulus, BV: Blood vein.*

Cardiac Histology

The histological analysis of the hearts of mice in the control (Fig. 10A) and treated groups with Dose1 (Fig. 10B) and Dose2 (Fig. 10C) indicated structure of myocardium stays unaffected following treatment: the cardio myocytes maintain their normal structure, the vascularization is intact, and no significant changes (necrosis, inflammatory infiltration, or cellular disarray) are identified. This histological stability suggests that the plant has no damaging or altering impacts on the structure and function of the heart muscle.

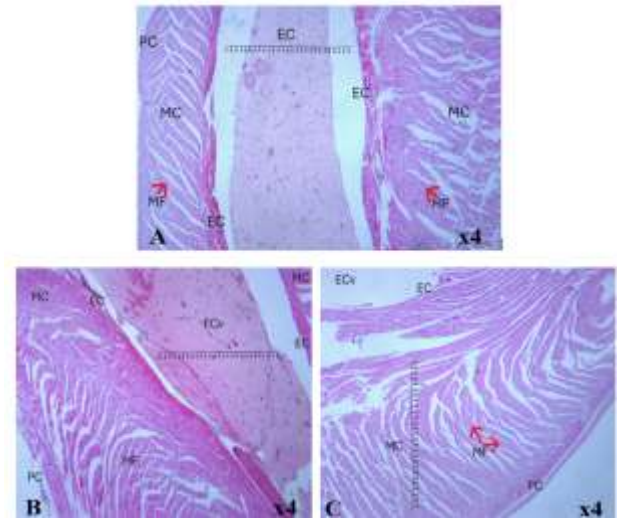

Figure 10. *Histological sections of the heart of control group and treated mice with S. tenacissima L. AQ extract. (hematoxyline & eosin , x4).* ***A****: control group,* ***B****: Treated group with 2 g/kg,* ***C****: Treated group with 5 g/kg. ECv: Ear cavity, MC: Myocardium, EC: Endocardium, PC: Pericardium, MF: Myocardial fiber.*

As this plant has a dose-dependent dangerous potential, since it induces liver damage and an increased inflammatory response. In contrast, *Cynodon dactylon* extracts (Poaceae family) show a great ability to protect and rebuild the liver in the presence of toxins [34]. This comparison emphasizes the significance of the distinctive characteristics of the extracts and doses used to evaluate the hepatoprotective or toxic effects of related plants.

4. Conclusions

This study was the first to report the acute toxicity, anti-inflammatory characteristics, and antioxidant activity of *S. tenacissima*, a species commonly found in Algeria. The results of the current study show that AQ extract has high antioxidant activity, inhibit inflammatory mechanisms, both *in vitro* and *in vivo* and a generally positive safety profile. However, the documented hepatic histological and haematological alterations at high doses indicate the importance of using caution. As a result, this plant may have potential as a naturally occurring source of bioactive substances with protective effects against oxidative stress and inflammatory diseases. However, the therapeutic use of this plant should be approached with caution and restricted to well-defined, regulated doses in order to reduce the possibility of subclinical toxicity.

Author Statements:

- **Ethical approval:** All experimental investigations were approved by the Algerian Association of Sciences in Animal Experimentation Committee (http://aasea.asso.dz/articles/) No.8808/1988, which deals with veterinary medical activities and animal health protection (No. JORA 004/1988).
- **Conflict of interest:** No conflicts of interest need to be reported.
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- **Author contributions:** Each author contributed to the conception and planning of this research. WT: Research, ideation, technique, formal analysis, and initial draft writing. AB: Oversight, verification, and approval of this article's final draft. Histological analysis of SG and AB. AB: Methodology, experimentation, and supervision. SD and NB: Methodology,

experimentation, and supervision. SS: Performing the biochemical and hematological analysis. SK: Validation, supervision, review, and editing. SD: Validation, supervision, review, and editing. All of the authors had read and approved the article's final version.

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- **Data availability statement:** upon request, the corresponding author may supply the data underlying the study's findings. The public cannot access the data due to ethical and privacy issues.

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