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RESEARCH ARTICLE

EVALUATION OF THE ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITIES OF THE HYDROETHANOLIC EXTRACT OF THE ROOT BARK OF SARCOCEPHALUS LATIFOLIUS

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ABSTRACT

Background: In Togo, *Sarcocephalus latifolius* is used in traditional medicine to treat various diseases including liver disease. **Objective**: To evaluate the antioxidant and hepatoprotective activities of the hydroethanolic extract of the bark of the root of *Sarcocephalus latifolius* (EHESL). **Methods**: After studying the phytochemical composition of the extract, the antioxidant power was evaluated by diphenyl-picryl-hydrazyl (DPPH) and iron reducing power (FRAP) tests. Hepatoprotective activity was assessed using a model of acute paracetamol-induced hepatotoxicity in rats. Sub-chronic toxicity and cytotoxicity (with the sea shrimp model) were evaluated. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by multiple Tukey comparison tests to assess significant differences between groups. The results were considered significant at $P<0.05$. **Results**: The sub-chronic toxicity study did not show signs of toxicity at doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg. The cytotoxicity assessment gave an LC50 = 1.88 mg/mL indicating that the extract is not cytotoxic. The extract showed antioxidant properties with an IC50 of 142.91 ± 1.96 µg/ml in the DPPH assay and a FRAP of 57.53 ± 0.54 µM Fe(II)/g of extract. Evaluation of the phytochemical composition revealed the presence of anthocyanins, tannins, flavonoids, saponins and terpenoids. Administration of the extract at doses 100 mg/kg and 300 mg/kg significantly prevented paracetamol hepatotoxicity compared to the distilled water group. Its results were similar to those obtained with Silymarin. **Conclusion**: The results show that the hydroethanolic extract of the root bark of *Sarcocephalus latifolius* contains bioactive substances with antioxidant and hepatoprotective activity.

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INTRODUCTION

The Rubiaceae family is very cosmopolitan, most of whose species are tropical, while herbaceous plants are more common in temperate zones. They are a family renowned for their wealth of alkaloids (1). It is extremely large and includes 500 genera and nearly 7000 species (2). The species *S. latifolius* is a shrub 5-6 m high, sometimes reaching 8-9 m, branching with a bushy, stunted stem with a tortuous bole 30 cm in diameter. The branches are flexible, lianescent, intertwined, erect and then drooping. The bark is very fibrous, the wood is white or yellow quite soft. It is distributed exclusively in tropical Africa, more particularly in sub-Saharan West Africa, extending from Senegal to Zaire, but is also found as far as eastern Sudan (Sudano-Guinean zone). Angola, Cameroon, Gabon, Nigeria, Uganda, Senegal, Sierra Leone, Sudan are some of the countries where this shrub can be found. It is a scattered, common and locally abundant species (3). It is very common in wooded savannahs, forest galleries and dry forest undergrowth in West Africa (Togo, Benin, Mali, Guinea, Senegal, Nigeria, etc.). *S. latifolius* is a plant widely used in traditional medicine to prevent and cure several pathologies (4). In order to validate this use, several studies have been conducted. The latter have shown different biological activities such as anti-inflammatory, antipyretic (5), antioxidant (6), as well as hepatoprotective activity (7). In Togo, *S. latifolius* is used in traditional medicine to treat various diseases including liver diseases (8). The aim of this work is to evaluate the pharmacological activity of the S. *latifolius* species from Togo. Thus, the work focused on the search for the main chemical groups and on the evaluation of the antioxidant and hepatoprotective activity, the sub-chronic toxicity and the

cytotoxicity of the hydroethanolic extract of the bark of the root of *S. latifolius*.

MATERIALS AND METHODS

Animal Material : Rats of the *Wistar* of both sexes used in the present study were bred in the animal facility of the Laboratory of Physiology and Pharmacology of Natural Substances of the Faculty of Science (FDS) and at the animal facility of the Pharmaceutical Sciences Laboratory of the Faculty of Health Sciences (FSS), University of Lomé, at room temperature with a 12-hour light/dark cycle. They have free access to food and water. Their weight was between 200 and 250 g.

Plant material and preparation of the extract: The fresh roots of *S. Latifolius* were harvested at Tsekpo dedekpo in the maritime region of southern Togo in July 2023. The bark was peeled from the roots and washed with tap water. They were air-dried under air conditioning for three days and were roughly powdered using an electric mill (Thomas Scientic Laboratory Mill Model 4, USA). The powder (100 g) was macerated at room temperature with 1 L of ethanol-water (8:2, v/v) for 72 h and the supernatant collected by filtration with filter paper. The solvent was removed from the filtrate using a rotary vacuum evaporator (Buchi, Japan) at 40 °C and the crude extract, which represents a yield of about 21% (w/w), was kept between +2°C and +8°C for the experiments.

Phytochemical screening : Major chemical groups such as alkaloids, condensed tannins, hydrolyzed tannins, anthocyanins, leukoanthocyanins, flavonoids, anthraquinones, saponins, and terpenoids have been determined by an approach based on solubility tests, precipitation and staining reactions according to the methods described in the literature (9).

Determination of total phenol contents : The total polyphenol content of the extract was determined by the Folin Ciocalteu method (10) with some modifications. To a volume of 1 ml of a solution of Folin Ciocalteu (diluted to 1/10) 200 µl of a solution of 1mg/ml of extract was added and the whole incubated at 2 min at room temperature. Then 800 µl of sodium carbonate at 75 g/l are added. The mixture was well homogenized and the whole was incubated for 30 min at room temperature away from light and the reading was carried out at 765 nm against a blank. The calibration curve was performed with gallic acid at concentrations between 0 and 100 µm/ml. Results were expressed in milligrams of gallic acid equivalent per gram of dry matter (mg EAG/g of extract).

Determination of flavonoid contents : The flavonoid content of the extracts was determined using the aluminum trichloride colorimetric method (11). To 2 ml of 2% $AlCl_3$ (m/v) in pure methanol, 2 ml of extract (1 mg/ml) is added also in methanol. The mixture was incubated for 10 min in the dark and absorbance was measured at 415 nm against a blank. The standard range of 25 to 100 µg/ml was prepared under the same conditions as the extract. The calibration curve was plotted using the different concentrations of quercetin and the results were expressed in milligrams of quercetin equivalent per gram of dry extract (mg EQ/g of extract).

Evaluation of antioxidant activity

DPPH Test : The free radical scavenging activity of the extract was evaluated using 2,2'-diphenyl-1 picrylhydrazyl (11) with some modifications. One hundred microliters (100 µL) of extract of different concentrations (25, 50 and 100 µg/ml) were added to 2 mL of DPPH (0.004% prepared in methanol). The mixture was homogenized and the absorbance reading was made with a spectrophotometer at 517 nm after 30 minutes of incubation at room temperature in the dark against the blank. Three tests were carried out for each concentration. The reference was quercetin (25 to 100 µg/ml) prepared under the same conditions. The results were expressed as a percentage of inhibition and then the equations of the corresponding regression lines were used to calculate the IC_{50}. The percent inhibition (%I) of free radical activity was calculated according to the formula:

$$\%I = \frac{\text{Abs (B)} - \text{Abs (E)}}{\text{Abs (B)}} \times 100$$

Abs (B) = Absorbance of white

Abs (E) = Absorbance of the sample

FRAP Testing : The revised protocol of Benzie and Strain (12) was used for the ferric antioxidant reducing potency (FRAP) test. The efficiency of this technique depends on the ability of the sample to convert ferrous tripyridyltriazine (Fe(II)-TPTZ) to ferric tripyridyltriazine (Fe(III)-TPTZ) at a low pH. The formation of Fe(II)-TPTZ gives a strong blue color, which can be read at 593 nm. Specifically, 100 µL of the extracts were combined with 2 mL of freshly prepared FRAP solution, which contained 25 mL of 300 mM acetate buffer (300 mM, pH 3.6), 2.5 mL of 2,4,6-tripyridyl-triazine (TPTZ) (10 mM) in HCl (40 mM) and 2.5 mL of H_{2O} for standard FRAP and 20 mM of ferric chloride solution {$FeCl_3.6H_2O$} for the FRAP of the sample. After 30 min of incubation in the dark, absorbance was measured at 593 nm. In the range of 0 to 100 µM $FeSO_4.7H_2O$, the standard curve was linear. A comparison was made between the results of the extract and those of ascorbic acid in terms of µM Fe(II)/g of dry plant extract.

Hepatoprotective activity : The study was carried out on 25 rats. The animals were randomly divided into 5 groups of 5 rats each: group I (control) served as a normal control, group II (PCM) served as a model of paracetamol-induced hepatotoxicity; both groups I and II were orally administered distilled water (10 ml/kg) once daily for 7 consecutive days, group III (Silymarin 100 mg/kg + PCM) served as the positive control group and received oral Silymarin (100 mg/kg) once daily for 7 consecutive days, and Groups IV and V (EHESL 100 mg/kg + PCM and EHESL 300 mg/kg + PCM) were administered EHESL orally, respectively, at 100 or 300 mg/kg once daily for 7 consecutive days. Two hours after the last administration of extract or Silymarin, groups II-V received a single oral dose of paracetamol (2g/kg). Twenty-four hours (24h) after paracetamol administration, blood samples were taken from fasting rats under partial anesthesia with ether oxide by retroorbital sinus puncture. The blood in the dry tubes was centrifuged at 4000 rpm for 10 min using an electric centrifuge (ROTOFIX 32 A *Hettich*), then the serum was decanted and frozen at -20°C for subsequent determination of total cholesterol (CHOL) and triglycerides (TG), transaminases (AST and ALT), alkaline phosphatase (PAL), total bilirubin (BILT) and direct bilirubin (BILD) and lactate dehydrogenase (LDH) using the cobas c311 automate and Roche/Hitachi reagents.

Evaluation of the toxicity of the extract

Sub-chronic toxicity : Twenty *Wistar* rats of both sexes were randomly assigned to four groups (five animals in each group), one control group, and three treatment groups. The vehicle or extract was administered daily (28 consecutive days) to control and EHESL-treated rats, respectively, for each dose (250, 500, and 1000 mg/kg) of body weight. The animals were observed at least twice daily for behavior change, morbidity, and mortality. The animals body weights were measured every 5 days. On day 29, blood samples were collected from fasting rats under partial anesthesia with ether oxide, then the rats were sacrificed and then dissected. Organ weights (heart, liver, kidney and spleen) were recorded separately.

Evaluation of Cytotoxicity : The cytotoxicity test was performed following the lethality test of the larvae of *Artemia salina* (13). Five hundred milligrams of *Artemia salina* larvae were incubated in 500 ml of seawater for 48 h with mechanical agitation. The larvae were then distributed in tubes of different concentrations (0.05-25 mg/mL). Each tube contained 16 larvae in 2 mL of solution. An eleventh tube also containing 16 larvae in 2 ml of seawater was used as a control. The tubes were placed under automatic agitation for 24 h and then observed to determine larval mortality, defined as larvae immobility for 30 seconds.

Statistical analysis : The results are expressed as an average ± standard error of the mean. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by multiple Tukey comparison tests to assess significant differences between groups. The results were considered significant at $P < 0.05$. All statistical analyses were performed using GraphPad Prism 8 from GraphPad Software, Inc. (California, USA) and Excel 2019.

RESULTS

Phytochemical screening and determination of the total phenol and flavonoid content of the extract : The bioactive compounds identified by the phytochemical analysis of the hydroethanolic extract of *S. latifolius* root bark were condensed and hydrolyzed tannins, flavonoids, saponins and terpenoids, anthocyanins, and flavonoids (Table 1).

Table 1: Phytochemical composition of *S. latifolius extract*

Groups	Results
Alkaloids	+
Condensed tannins	+
Hydrolyzed tannins	+
Anthocyanins	+
Leukoanthocyanins	-
Flavonoids	+
Anthraquinones	-
Saponins	+
Terpenoids	+

(+) : Presence ; (-) : Absence

Total phenol and flavonoid concentrations were determined from the linear regression curves for phenols and flavonoids, respectively ($y = 0.0006277x + 0.06307$; $R^2 = 0.9902$ and $y = 0.0005405x + 0.2366$; $R^2 = 0.9718$). The total phenol contents obtained were 38,125 ± 0.369 mg EAG/g of extract. The flavonoid levels obtained were 55.648 ± 0.884 mg EQ/g extract (Table 2).

Table 2: Total polyphenol and flavonoid content of *S. latifolius* extract

Chemical compounds	Chemical compound contents
Flavonoids	55.648 ± 0.884 mg EQ/g extract
Polyphenols	38.125 ± 0.369 mg EAG/g extract

Table 3. Antioxidant activity of *S. latifolius extract*

	DPPH (IC_{50}) µg/mL	FRAP (µM Fe(II)/g)
S. latifolius	142.91 ± 1.96	57.53 ± 0.54

Evaluation of antioxidant activity

DPPH test : The 25 µg/mL extract inhibited the DPPH radical at a percentage of 13.87 ± 3.56%. This inhibition was 20.23 ± 3.31% for the 50 µg/ml extract and 34.56 ± 3.12% for the 100 µg/ml extract. Quercetin radical inhibition was 32.07 ± 4.23%, 57.68 ± 5.28%, and 83.23 ± 4.83% at 25, 50, and 100 µg/mL, respectively (Table 3). The IC_{50} of extract and quercetin were determined from the linear regression curves for extract and quercetin, respectively ($y = 0.3311x + 2.6813$; $R^2 = 0.9706$ and $y = 0.8179x + 7.7179$; $R^2 = 0.9549$). The IC_{50} of the extract obtained was 142.91 ± 1.96 µg/ml and that of quercetin was 51.70 ± 4.45 µg/ml (Table 3).

FRAP Testing : The total antioxidant activity of *Sarcocephalus latifolius* root bark extract was 57.53 ± 0.54 µM Fe(II)/g (Table 3). Ascorbic acid, which was the positive control, measured 97.37 ± 0.86 µM Fe(II)/g.

Evaluation of hepatoprotective activity: The effect of the extract at doses of 100 and 300 mg/kg was studied on serum enzyme markers of liver damage, cholesterol, triglycerides and bilirubins in rats intoxicated with paracetamol. Compared to the control group (Group I), paracetamol administration (Group II) induced a significant increase in serum markers of liver injury, including ALT and LDH ($P < 0.0001$), AST ($P < 0.001$), PAL ($P < 0.05$) (Table 4), total and direct bilirubin ($P < 0.01$), cholesterol and triglycerides ($P < 0.05$) (Table 5). However, pretreatment with the hydroethanolic extract of *S. latifolius* root bark significantly reduced serum concentrations of markers of liver injury (Table 4, 5). The effect of EHESL on paracetamol-induced acute hepatitis is significant at doses of 100 and 300 mg/kg resulting in a reduction in serum levels of ALT ($P < 0.001$), AST and LDH ($P< 0.0001$), PAL ($P < 0.05$ and $P < 0.01$) respectively (Table 4) and cholesterol ($P< 0.05$) (Table 5). However, the decrease in triglyceride and total bilirubin (Table 5) was significant only at 300 mg/kg ($P <0.05$ and $P < 0.01$, respectively). Silymarin-treated animals also showed significant protection ($P < 0.0001$, $P < 0.001$, $P < 0.01$, $P < 0.05$) against paracetamol-induced liver injury through the markers mentioned above (Table 4.5).

Toxicity assessment

Sub-chronic toxicity

Effect of oral administration of hydroethanolic extract of *S. latifolius* root bark on the variation in rat weight over 28 days : The curve in Figure 1 shows a regular evolution of weight in the groups except for the dose of 1000 mg/kg were

Table 4: Effect of the extract on enzyme markers of liver damage

Parameters	Control (ED)	CFM (2g/kg)	Sylimarine (100 mg/kg)	EHESL (100 mg/kg)	EHESL (300 mg/kg)
AST	178.7 ± 14.6	4044.9 ± 1155.4^{e}	651.7 ± 316.1^{f}	429.1 ± 183.7^{f}	335.4 ± 183.7^{f}
ALT	35.5 ± 2.9	4337.1 ± 789.4^{g}	687.9 ± 425.6^{h}	487.6 ± 323.8^{h}	284.9 ± 253.5^{h}
LDH	2267.8 ± 183.8	11860.6 ± 1540.6^{g}	2392.7 ± 355.2^{h}	2923.5 ± 388.1^{h}	2417.75 ± 230.0^{h}
PAL	66.6 ± 6.3	94 ± 4.8^{a}	68.2 ± 13.1^{d}	68.0 ± 10.8^{b}	58.2 ± 4.8^{d}

ED: Distilled water, PCM: paracetamol, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, PAL: Alkaline phosphatases, LDH: Lactate dehydrogenase. a $p < 0.05$ compared to the control group ; b $p < 0.05$ compared to the PCM group ; c $p < 0.01$ compared to the control group ; d $p < 0.01$ compared to the PCM group ; e $p < 0.001$ compared to the control group ; f $p < 0.001$ compared to the PCM group ; g $p < 0.0001$ compared to the control group ; h $p < 0.0001$ compared to the PCM group.

Table 5. Effect of the extract on cholesterol, triglycerides and bilirubins

Parameters	Control (ED)	CFM (2g/kg)	Sylimarine (100 mg/kg)	EHESL (100 mg/kg)	EHESL (300 mg/kg)
CHOL	0.38 ± 0.05	0.70 ± 0.05^{a}	0.39 ± 0.10^{b}	0.42 ± 0.05^{b}	0.40 ± 0.07^{b}
TRIGLY	0.58 ± 0.03	0.86 ± 0.03^{a}	0.45 ± 0.03^{e}	0.64 ± 0.09	0.48 ± 0.06^{d}
BILT	0.91 ± 0.03	2.54 ± 0.35^{c}	1.41 ± 0.29^{b}	1.68 ± 0.18	1.55 ± 0.10^{b}
BILC	0.40 ± 0.07	1.28 ± 0.14^{c}	0.64 ± 0.03	1.03 ± 0.36	0.67 ± 0.06

ED: Distilled water, PCM: paracetamol, CHOL: Total cholesterol, TRIGLY: Triglycerides, BILT: total bilirubin, BILC: conjugated bilirubin. a $p < 0.05$ compared to the control group ; b $p < 0.05$ compared to the PCM group ; c $p < 0.01$ compared to the control group ; d $p < 0.01$ compared to the PCM group ; e $p < 0.001$ compared to the PCM group.

Table 6. Organ weights after the experiment

Organs	Control (ED)	EHESL (250 mg/kg)	EHESL (500 mg/kg)	EHESL (1000 mg/kg)
Liver	7.26 ± 0.72	9.02 ± 0.62	9.36 ± 1.47	9.66 ± 1.12
Kidney	1.26 ± 0.04	1.51 ± 0.14	1.71 ± 0.17	1.68 ± 0.34
Heart	0.89 ± 0.18	0.97 ± 0.15	0.92 ± 0.11	0.91 ± 0.10
Spleen	0.68 ± 0.09	0.72 ± 0.16	0.81 ± 0.09	0.68 ± 0.12

No significant difference between the different groups

Table 7. Results of the effect of the extract on selected biochemical parameters

Parameters	Control (ED)	EHESL (250 mg/kg)	EHESL (500 mg/kg)	EHESL (1000 mg/kg)
ASAT	176.8 ± 26.7	165.3 ± 19.8	155.1 ± 18.0	153.8 ± 31.1
ALT	54.9 ± 12.1	59.4 ± 11.3	69.0 ± 9.4	70.2 ± 15.6
STAKE	106.3 ± 11.4	99.2 ± 16.3	78.6 ± 9.6	111.3 ± 20.8
BILT	0.60 ± 0.15	0.81 ± 0.14	0.10 ± 0.10	0.84 ± 0.11
BILC	0.45 ± 0.17	0.49 ± 0.10	0.58 ± 0.08	0.52 ± 0.06
CK	2055.5 ± 570.5	2055.8 ± 674.5	1676.8 ± 414.2	1875.0 ± 773.5
CK-MB	3180.4 ± 424.8	2762.2 ± 544.6	2428.9 ± 443.3	2200.6 ± 366.4*
Urea	0.27 ± 0.05	0.32 ± 0.06	0.30 ± 0.04	0.38 ± 0.07
Creatinine	0.30 ± 0.08	0.40 ± 0.07	0.41 ± 0.06	0.53 ± 0.05***
Glucose	0.93 ± 0.15	0.94 ± 0.11	0.97 ± 0.07	1.29 ± 0.22*
Cholesterol	0.52 ± 0.13	0.59 ± 0.09	0.51 ± 0.05	0.40 ± 0.06
Triglycerides	0.47 ± 0.14	0.54 ± 0.14	0.54 ± 0.14	0.37 ± 0.21
Protein	75.0 ± 1.1	76.9 ± 3.9	77.2 ± 2.4	77.5 ± 3.0

ED (distilled water). Comparisons were made with the control group. * Significant difference. *** High difference

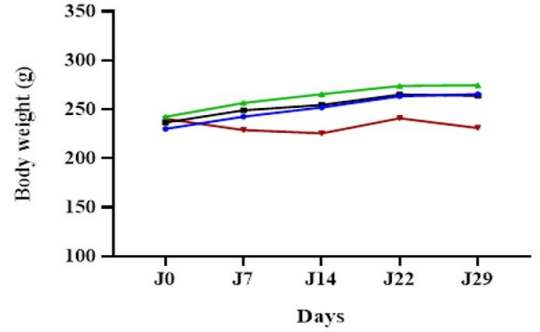

Figure 1. Curve of variation in rat weight during sub-chronic toxicity

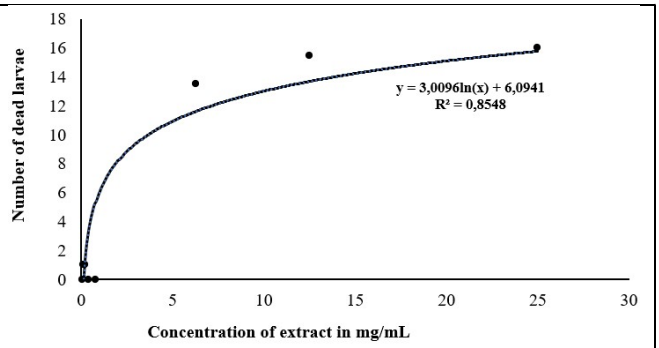

Figure 2. Curve expressing the number of dead larvae as a function of concentration

there is a non-regular evolution. Analysis of the results showed no significant difference between the groups

Effect of oral administration of hydroethanolic extract of *S. latifolius* root bark on organ weights : Table 6 gives us a summary of the analysis of the organ weights of rats collected from each group of rats after sacrificing them. The results were not significant

Effect of oral administration of hydroethanolic extract of *S. latifolius* root bark on biochemical parameters : The results of the effect of the extract on the biochemical parameters tested showed that at the dose of 1000 mg/kg there was a significant increase in the concentrations of creatinine ($P < 0.001$) and glucose ($P < 0.05$), and a significant decrease in CK-MB ($P < 0.05$) (Table 7).

Cytotoxicity : We used the number of dead larvae in each tube to plot a logarithmic trend line (Figure 2) that expressed the number of dead larvae as a function of the concentration of the solutions ($y = 3.0096\ln(x) + 6.0941$ with $R^2 = 0.8548$). From this equation, we determined the lethal concentration 50 (LC_{50}) which was 1.88 mg/mL.

DISCUSSION

The purpose of this study is to evaluate the antioxidant and hepatoprotective activities of the hydroethanolic extract of the root bark of *S. latifolius*, a medicinal plant widely used in Africa. Prior to the studies on antioxidant and hepatoprotective activity, a phytochemical screening was carried out to determine the presence of certain chemical groups in the plant extract. Qualitative phytochemical tests have shown the presence of certain chemical groups such as alkaloids, flavonoids, tannins, terpenoids, anthocyanins, and saponins in the hydroethanolic extract of the root bark of *S. latifolius.* These results are consistent with previous studies on the methanolic extract of the root bark and leaves of *S. latifolius* (14). In order to evaluate the antioxidant power of the extract, we performed the DPPH test and the FRAP test. The reducing power of DPPH may be due to its ability to give electrons or hydrogen (15). The antioxidant activity of hydroethanolic extract of *S. latifolius* could be attributed to its flavonoid and polyphenol content. Flavonoids act as scavengers of various oxidizing species, namely the superoxide anion, hydroxyl radical or peroxy radicals. They also act as singlet oxygen absorbers (16). Flavonoids do not necessarily act as antioxidants. Some have the ability to stimulate the degradation of deoxyribose as a result of the reduction of Fe^{3+}. These flavonoids have pro-oxidant properties, probably through the redox of iron (17). The hydroxyl groups of polyphenols have the ability to scavenge radicals (18). Phenolic compounds have also been shown to be effective hydrogen donors. As such, they are excellent antioxidants. (19)
For the study of the hepatoprotective activity of EHESL, the paracetamol-induced hepatotoxicity model was used because it is a promising experimental model to evaluate the hepatoprotective effect of plant extracts because the use of a single dose was sufficient to induce hepatotoxicity (20). NAPQI, a metabolite derived from the oxidation of paracetamol by CYP 450, is one of the toxins eliminated by the liver through its conjugation with the antioxidant GSH. The accumulation of NAPQI induces the depletion of the antioxidant thus leading to hepatotoxicity by several mechanisms, among them lipid peroxidation (21), oxidative stress accompanied by mitochondrial dysfunction, and ultimately leading to hepatocellular death (22). The hepatotoxicity of paracetamol followed by the events mentioned above, leads to an alteration of mitochondrial respiration, the formation of reactive oxygen species (ROS) and oxidative stress (23).

In the present study, general indicators of paracetamol-induced hepatotoxicity, including AST, ALT, PAL, and LDH activities, as well as serum bilirubin, cholesterol, and triglyceride levels were evident. However, pretreatment with EHESL inhibited the leakage of liver enzymes into the systemic circulation in rats. These results suggest that EHESL significantly suppressed paracetamol-induced liver damage through the recovery of liver cell function and structure. Indeed, in a liver injury, the function of hepatocytes is disrupted, causing an increased enzyme level in the serum (24). This increase is due to an increase in the fragility of the plasma membrane of hepatocytes, which leads to the release of intracellular enzymes into the bloodstream (25). Elevation of serum alkaline phosphatase, which is normally released into the bile, may result from damage or obstruction of the bile ducts (26).
Lactate dehydrogenase is a marker of cell degeneration, used to assess liver function. The increase in the level of this intracellular enzyme in the serum of poisoned rats reflects the damage to liver cell membranes (27). Elevated bilirubin in the bloodstream could be attributed to elevated synthesis, increased hemolysis, decreased conjugation, or impaired transport (28). And therefore, bilirubin is used as an indicator to assess the secretory function of hepatocytes (29). Triglyceride and cholesterol levels increased significantly under the effect of paracetamol. This indicates the presence of disturbances in the mechanisms of association of triglycerides with the appropriate apoprotein to form the lipoprotein carrier molecule (30). Treatment with the extract reduces the level of transaminases, PAL, LDH. This indicates that the extract protects hepatocytes against free radical attacks and decreases intracellular enzyme leakage, preserving the plasma membrane. Indeed, it has been reported that plant extracts such as *A. americana* decrease the levels of liver transaminases, PAL and bilirubin in rats poisoned by paracetamol (31). Similar reports from a few other plants, such as *Deinococcus radiodurans* (32) and *Lumnitzer aracemosa* (33) have also been noted. Treatment with *S. latifolius* extract reduces triglyceride and cholesterol levels. These results are consistent with a study that showed that the ethanolic extract of *Ceriops decandra* leaves decreases triglyceride and cholesterol levels in liver damage (34). Peroxidation of membrane lipids also leads to the release of transaminases (ALT, AST), PAL, bilirubin and LDH (35). The hepatoprotective effect of the plant extract appears to be due to the free radical-scavenging properties, which leads to the inhibition of lipid peroxidation and the preservation of the cell membrane (36).

The effects of *S. latifolius* extract are similar to those of Silymarin, which is used as a reference hepatoprotector. The hepatoprotective activity of Silymarin is associated with its ability to act as a radical scavenger, thus protecting membrane permeability (37). To determine the safety of *S. latifolius*, subchronic toxicity and cytotoxicity were studied. According to an earlier study (38), the assessment of the toxic effect by observation of the general behaviour of the animals and the difference in weight are some of the critical parameters for early signs of toxicity. In the present study, no general

behavioural changes were noted in rats treated with the extract compared to control rats for 28 days of observation. Similarly, no abnormalities in weight were noted. However, at the dose of 1000 mg/kg, the effect of the extract on a few biochemical parameters showed a decrease in the concentration of CK-MB and an increase in glucose and creatinine concentrations. The sea shrimp lethality test is a preliminary, rapid, and comprehensive toxicity test that is used to check the cytotoxicity of chemicals. The test revealed that the hydroethanolic extract of the root bark of *S. latifolius* was safe because the calculated LC50 was greater than 0.1 mg/mL, the maximum value above which an extract can be considered non-toxic. These results are consistent with the result of a previous study (39).

CONCLUSION

In the present work, the antioxidant and hepatoprotective properties of the hydroethanol extract of *S. latifolius* are evaluated and the results are conclusive. The present work proves that the use of *S. latifolius* in folk medicine for the treatment of various diseases whose liver diseases is justified.

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Abbreviations

ALT: Alanine aminotransferase
ANOVA: Unidirectional Analysis of Variance
AST: Aspartate aminotransferase
CHOL: Total cholesterol
DPPH: Diphenyl-picryl-hydrazyl
ED: Distilled water
EHESL: Hydroethanolic extract from the root bark of *Sarcocephalus latifolius*
FRAP: Ferric reducing ability of plasma
GSH: Reduced Gluthation
LDH: Lactate Dehydrogenase
NAPQI: N-acetyl-p-benzoquinone imine
PAL: Alkaline Phosphatases
PCM: Paracetamol
ROS: Reactive Oxygen Species
S. latifolius: Sarcocephalus latifolius
TG: Triglycerides

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