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Preclinical Evaluation of the Antidiabetic Effect and Phytochemical HPLC-MS ESI-QTOF Analysis of *Sclerocarya birrea* (A. Rich) Hoscht Bark of Trunk Aqueous Extract in Alloxan-Induced Diabetic Wistar Rat

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Abstract

Introduction: Diabetes is a serious public health problem requiring complex treatment. Numerous ethnopharmacological studies have reported the traditional use of *Sclerocarya birrea* in managing diabetic patients. This study aims to demonstrate, preclinically, the antidiabetic effects of the aqueous decoction of *S. birrea* trunk bark. **Methods**: Phytochemical analysis was performed by HPLC-MS. The effects of the extracts (Sb5 and Sb25) and 0.9% NaCl on the normal blood glucose levels of the animals were determined. Diabetes induction was performed intraperitoneally by administering a single dose of alloxan (150 mg/kg) in normoglycemic rats. The antidiabetic effects of the extracts (Allox + Sb5, Allox + Sb25) and glibenclamide (Allox + Glib5) were determined in Alloxan-induced diabetic animals for four weeks. **Results**: Interpretation of mass spectra obtained by HPLC-MS allowed the tentative identification of vanillic acid-4-sulfate and rhamnetin in Sb extract. Investigated doses of Sb extract showed an antidiabetic impact similar to the reference, glibenclamide, with a return to normal blood glucose in all treated rats only

after 4 days of treatment. Furthermore, Sb extract treatments reduced weight loss in diabetic rats. Sb had no negative impact on the balance of total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). **Conclusion**: The present study demonstrated the antidiabetic efficacy and, to some extent, the beneficial effects of Sb extract on Alloxan-induced diabetic rats' health. Detection of antidiabetic phytochemicals such as vanillic acid-4-sulfate and rhamnetin would justify this pharmacological property of the aqueous decoction of *S. birrea* trunk bark.

Keywords

Preclinical Study, *Sclerocarya birrea*, Antidiabetic Effect, Alloxan, HPLC-MS

1. Introduction

Diabetes is a non-communicable disease resulting from high levels of glucose in the blood, often responsible for serious damage to the heart (infarction), blood vessels (arteriosclerosis, stroke, etc.), eyes (retinopathy), kidneys (nephropathy) and nerves (neuropathy). The classification of diabetes has undergone several modifications, so by consensus, diabetes is diagnosed following glucose intolerance materialized by a fasting blood glucose level ≥ 126 mg/dL (7.0 mmol/L). Since then, several types of diabetes have been considered, depending on their specificity: fragile, insulin-resistant, gestational, pancreatic, endocrine, and iatrogenic [1]. According to the IDF Diabetes Atlas, this pathology affects 537 million adults (20 - 79 years) across the world, which is expected to reach 643 million (*i.e.*, 10.5%) by 2030 and 783 million (*i.e.*, 12.2%) by 2045. Unfortunately, more than 3/4 of adults with diabetes live in low- and middle-income countries, with one death every 5 seconds, according to 2021 statistics [2]. According to the WHO, nearly 24 million people were living with diabetes in Africa in 2021, and this figure is expected to reach 55 million by the year 2045, a potential increase of 129% [2]. Burkina Faso's prevalence was estimated at 4.9% in the general population [3], 13.9% in urban areas, and 5.7% in rural areas [4] [5]. According to a study by the Global Burden of Diseases, type 2 diabetes, in correlation with its complications, is responsible for a significant increase in cases of disability (22%) over the last ten years [6]. Once declared, the medical treatment of type 2 diabetes is based on antibiotic drugs, compliance with health and diet measures, monitoring of blood pressure parameters, and the lipid profile of patients [7]. Also, it is known that managing this pathology without complications is relatively expensive for the population in middle- and low-income countries [8]. Therefore, in addition to the conventional pathology management, numerous studies have shown that plant species would have good efficacy and safety of use both preclinically [9] [10]. Other studies have already shown that plant extracts are used in traditional settings to regulate blood sugar levels [11] [12]. With this aim, research on plant

extracts with potential antidiabetic properties is taking on significant proportions [13] [14]. This is the case of *Sclerocarya birrea* (A. Rich) Hoscht (Anacardiaceae), widely used in Burkina Faso's traditional environments to regulate hyperglycemia problems and was chosen in this study. Previous studies have shown that plant extract possesses various properties, including antioxidant, anti-microbial, anti-inflammatory, and anti-aging [13]. Other work has shown that extracts of *S. birrea* could protect striatal neurons and movement-associated functionalities in rats with diabetes-induced locomotion dysfunction [15]. An old study also showed the anti-inflammatory, analgesic, and antidiabetic properties of the plant's methanolic/methylene chloride extract in a rat model made diabetic by streptomycin [11]. However, to our knowledge, no antidiabetes activity of the used extract in diabetic rat models induced by alloxan has yet been achieved. Hence, the interest of the present study, which aims to evaluate, preclinically, the anti-hyperglycemic properties of the aqueous decoction of the trunk bark of *S. birrea* (marula) in Wistar rats made diabetic by alloxan.

2. Material and Methods

2.1. Material

2.1.1. Collection of Plant Material

The bark of the trunk of *Sclerocarya birrea* was collected in the locality of Loumbila, located 20 km northeast of the center of Ouagadougou and 7 km southwest of Ziniaré (provincial capital, Burkina Faso). After harvest, the samples were rinsed, dried away from sunlight, and made into powder using a mechanical grinder. A sample was collected and deposited at the herbarium of the National Centre for Scientific and Technological Research under the same number N360.

2.1.2. Experimental Animals

Adult male rats of Wistar strains, 12 weeks old and weighing 347.33 ± 9.79 g, were used in this study. These animals were obtained from CIRDES and then acclimated at the IRSS animal facility for one week in polycarbonate cages with wood shavings as bedding before use. They were fed wheat meal (29%) with free access to running water under standard conditions of temperature (23˚C ± 2˚C) with relative humidity (60% - 70%) followed by a 12 h light/dark cycle. All experiments were carried out following the procedures of the Guide to Good Practice in Animal Experimentation under the Declaration of Helsinki, which is in line with the terms of the Local Ethics Committee of Joseph KI-ZERBO University (Protocol number: CE-UJKZ/2024-05).

2.1.3. Chemicals Material

Alloxan monohydrate and NaCl were obtained from Sigma Aldrich Company (Merck KGaA, Darmstadt, Germany). Total cholesterol, HDL-cholesterol, LDL-cholesterol, and Triglycerides were from SPINREACT (Sant Esteve de Bas, Gérone, Espagne). Neu's reagent, $FeCl_3$, Sulfuric anisaldehyde, and Liebermann Bürchard's reagent were from Sigma Alrich (France).

2.2. Methods

2.2.1. Aqueous Decoction Extract Preparation

100 g of plant bark powder was diluted in 350 mL of distilled water, boiled for 25 minutes, and then cooled to room temperature. After cooling, the mixture obtained was centrifuged at 6000 rpm for 5 minutes. The aqueous decoction supernatant was collected, frozen, and freeze-dried. The lyophilized was then used for the experiments.

2.2.2. Phytochemical Study: LC-MS Screening

First, 1 mg of the Sb extract was dissolved in 10 mL of methanol and filtered. LC-MS analysis was carried out using an HPLC chain (Agilent Technologies, series 1290, Agilent, San Jose, CA, USA) equipped with a G7104A quaternary pump and G7167B autosampler. This HPLC chain was coupled to a mass spectrometer (Agilent Technologies, model 6545, San Jose, CA, USA) equipped with a continuous-jet electrospray ionization source (ESI source). Negative-mode ionization was applied to detect phenolic compounds with a set pressure limit of 0 - 1300 bar. Spectra were recorded in negative ionization mode. 1 µL of the Sb extract solution was loaded onto an injection column for compound separation. The LC-MS had a C18 column (2.1 × 50 mm, internal diameter 1.8 µm; brand Zorbax RRHD Eclipse Plus C18, Agilent, USA). The mobile phase comprised solvent A (acidified water + 0.1% formic acid) and solvent B (100% acrylonitrile). Chromatography was performed under gradient conditions. The mobile phase was set for a continuous flow of 0.1 mL/min for 15 min. The gradient program shown in Table 1 was applied.

Table 1. LC-MS chromatography mobile phase gradient conditions.

Time (min)	Solvent A (Water + Formic acid 0.1%)	Solvent B (Acrylonitrile 100%)
0.00	70.00	30.00
5.00	70.00	30.00
7.00	40.00	60.00
8.00	38.00	62.00
8.50	38.00	62.00
10.00	30.00	70.00
12.00	30.00	70.00
15.00	70.00	30.00

2.2.3. Antidiabetic Efficacity Assessment

1) Diabetes induction

Type 2 diabetes was induced according to the methods described by [16] Diniz *et al.*, 2008 and that of Banda *et al.* 2018) with slight modifications [17].

Briefly, male Wistar rats fasted on a water diet for 12 hours, and fasting blood sugar was determined with a range of 60 - 120 mg/dL considered normal. A solution of monohydrate alloxan in NaCl 0.9% was prepared extemporaneously and

then administered to the animals intraperitoneally (dose of 150 mg/kg body weight). A comparable volume of 0.9% NaCl was administered to control animals. A glucose solution (10%) was added to the water to prevent hypoglycemia attacks. Seventy-two hours (72 h) after the alloxan injection, all animals' fasting blood sugar levels were determined, and only those with blood sugar levels greater than or equal to 200 mg/dL were considered diabetic.

2) Administration of tests substances

After the induction of diabetes, seven (7) groups of six (6) homogeneous rats each, including four (4) groups from diabetic rats and three (3) groups from normal rats, were formed. The administration of the substances was carried out according to the following design:

Group 1 = Control: Administration of NaCl 0.9% alone for four weeks.

Group 2 = Allox: Single administration of Alloxan 150 mg/kg bw and followed for four weeks.

Group 3 = Sb5: Administration of Sb 5 mg/kg/day alone for four weeks.

Group 4 = Sb25: Administration of Sb 25 mg/kg/day alone for four weeks.

Group 5 = Allox + Glib: Single administration of Alloxan 150 mg/kg bw + Administration of glibenclamide 5 mg/kg/day for four weeks.

Group 6 = Allox + Sb5: Single administration of Alloxan 150 mg/kg bw + Administration of Sb 5 mg/kg/day alone for four weeks.

Group 7 = Allox + Sb25: Single administration of Alloxan 150 mg/kg bw + Administration of Sb 25 mg/kg/day alone for four weeks.

At the end of the experimental period and 24 hours after the last treatment, the different animals were anesthetized, and blood was collected by cardiac puncture. Then, they were humanely sacrificed, and the heart, liver, kidneys, spleen, and testicles were removed. Serum was collected for biochemical analyses. The organs were preserved for subsequent histopathological analyses.

3) Glycemia determination

Following the first measurement, the glycemia of each animal was determined every 04 days by the caudal puncture method for 28 days using a glucometer (brand). After each sample, the animal's tail was disinfected using alcohol.

4) Body weight, Water, and food intake measurement

The effects of Sb5, Sb25, Allox, Allox + Sb5, Allox + Sb25, Allox + Glib, and physiological water (NaCl 0.9%) on the evolution of body weight, water, and food intake during the study were determined.

For that, the body weights of all animals were recorded at inclusion and were divided into seven homogeneous batches of six (06) animals, including a control batch. During the experiment, the average weight of each batch was recorded with a periodicity of 4 days until the 28th day using an electronic scale.

Water and food consumption per lot of animals was measured at inclusion, followed by a periodic interval of four (04) days throughout the experiment.

5) Blood sample analysis

At the end of the experiment, the animals were humanely sacrificed. Blood was

drawn, and serum was collected after centrifugation at 4000 rpm for 10 min and stored at −20˚C. These samples were then used to determine total cholesterol (TC), low-density lipoprotein (LDL), triglycerides (TG), and high-density lipoprotein (HDL) levels.

6) Statistical analysis

All data collected during the experiment were subjected to statistical analysis using Prism 8.01 software. Results were expressed as mean ± SEM for all the experimental groups following one-way analysis of variance (ANOVA) with the Bonferroni multiple post-test. A statistically significant difference was considered when the p-value < 0.05.

3. Results

3.1. Chromatogram of Sb Extract

LC-MS analysis of Sb showed the presence of compounds materialized by the spectrum shown in Figure 1. This chromatogram comprises seven peaks appearing at different times (min) as shown in Figure 1. The height of each peak is proportional to its abundance in the Sb extract. Mass spectra analyzed the majority peaks. These peaks appear at retention time (RT) of 0.733 min and 13.668 min.

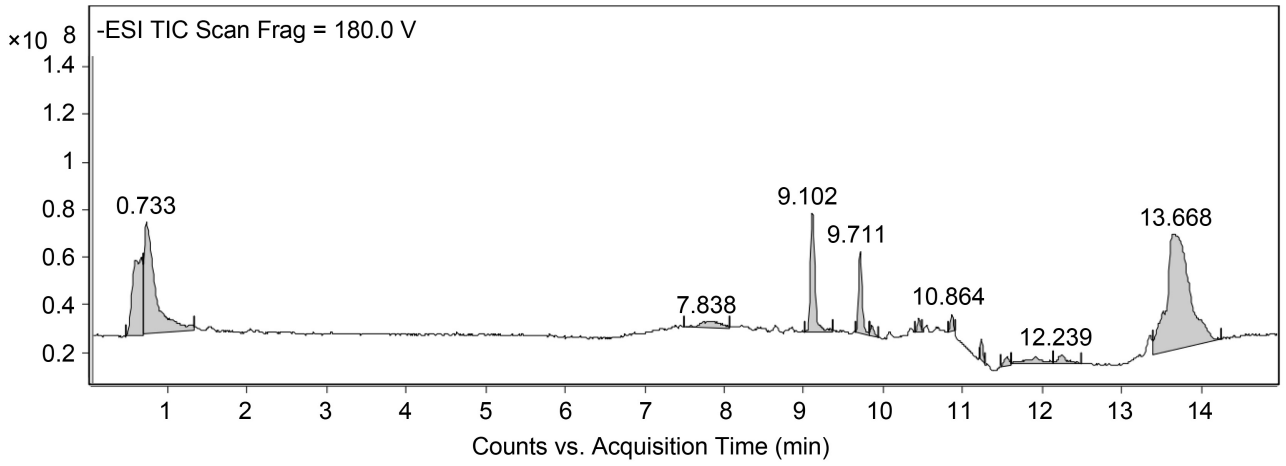

Figure 1. Chromatogram HPLC-MS of *S. birrea* trunk bark decoction.

3.2. Natural Molecules Identification

Figure 2 and Figure 3 show, respectively, the mass spectra of the majority peaks RT = 0.733 min and RT = 13.668 min of the Sb extract obtained by high-performance liquid chromatography with mass spectrometer (HPLC-MS) in negative electrospray ionization (ESI) mode. Analysis of these mass spectra identified a pseudomolecular ion for each mass spectrum and fragment. The pseudomolecular ion of peak RT = 0.733 was tentatively identified by comparing the experimental or measured mass (m/z = 247.0096), theoretical mass (m/z = 247.9990), and MS fragmentation pattern obtained from HPLC-MS analysis in negative ESI mode with compound data in various databases such as ChemSpider, Pubchem,

Dictionary of Natural Products and Phenol explorer. The compound identified is vanillic acid 4-sulfate. However, this identification remains provisional pending elucidation of the fragmentation process, taking into account losses and analysis of ionic charges. As previously described, the pseudo molecular ion of the RT = 13.668 min peak was identified by applying the same process described above.

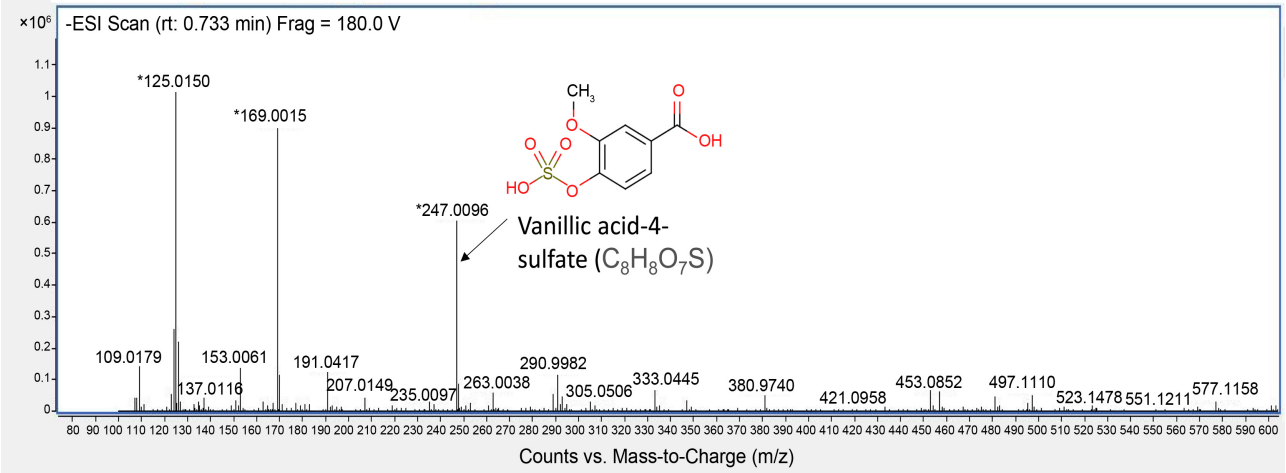

Figure 2. Mass spectrum of *S. birrea* trunk bark decoction at retention time 0.733 min.

Following the same procedure as above, rhamnetin (7-methyl-quercetin) was obtained by comparing the measured mass (m/z = 316.9304), the theoretical mass (m/z = 316.0583), and the fragmentation pattern (**Figure 3**).

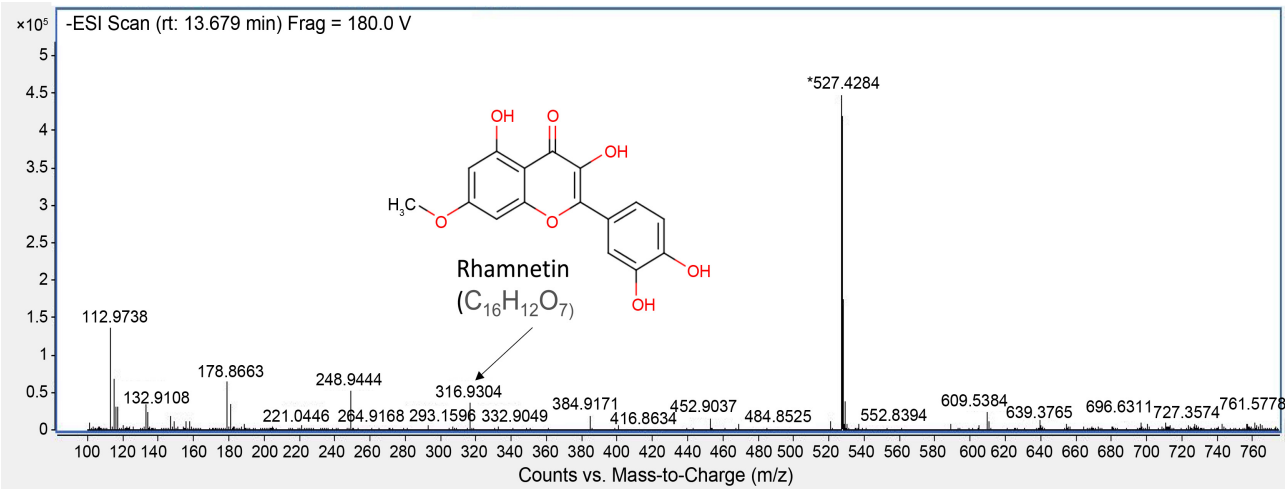

Figure 3. Mass spectrum of *S. birrea* trunk bark decoction at retention time 13.679 min.

3.3. Others Compound Identification

Other compounds were tentatively identified by comparing the experimental data obtained with data from previous work on the *S. birrea* species. However, the identification process for these compounds did not include the mass loss process. **Table 2** shows the compounds provisionally identified with MS fragmentation.

Table 2. Compounds tentatively identified in *Sclerocarya birrea* Sb extract.

N°	RT	Exp. m/z [M-H]	Theo. m/z [M-H]	Proposed compounds
1	0.7330	729.1261	729.1461	(Epi)catechin-(Epi)catechin-3'-O-gallate
2	0.7330	897.1323	897.1520	(Epi)catechin-3'-O-gallate -(Epi)gallocatechin-3'-O-gallate
3	9.1020	758.0358	757.2190	Cyanidin-3-O-glucosyl-rutinoside
4	9.7110	250.1291	250.1216	Ferruloylglycine
5	10.8600	630.9413	630.1320	Pelargonidin-3,5-O-diglucoside
6	10.8600	694.9371	694.2107	1,2-Diferuloylgentiobiose
7	13.6790	452.9037	452.13	Catechin3-O-glucose
8	13.6790	609.5484	609.1611	(Epi)gallocatechin dimer
9	13.6790	761.5778	761.1359	Bis(epi)galocatechin monogallate
10	13.6790	527.4284	527.1406	Guaiacylglycerol glucose gallate
11	13.6790	609.5374	609.1244	(Epi)gallocatechin dimer Peonidin-3-O-6p-coumaroyl-glucose

Abbreviation: RT = Retention Time; Exp. = Experimental; Theo = Theoric.

3.4. Clinical Course

The survival rate of the animals in the different groups used during this experiment is presented in Table 3. The results showed that no animal was lost in the different groups studied (100%) except for the group Allox, in which 2 of the six animals (33.33%) died within seven days. It should be noted that these lost animals were successfully replaced to reach the number of six.

Table 3. Survival rate of animals during the study.

Groups (n = 6)	Initial Total per group	Survivors number	Deaths number	Survival rate (%)
Control NaCl 0.9%	6	6	0	100
Allox	6	4	2	66.66
Sb5	6	6	0	100
Sb25	6	6	0	100
Allox + Glib	6	6	0	100
Allox + Sb5	6	6	0	100
Allox + Sb25	6	6	0	100

Abbreviation: Allox = Alloxan; Sb5: Decoction of *S. birrea* 5 mg/kg; Sb25: Decoction of *S. birrea* 25 mg/kg; Allox + Glib: Alloxan + Glibenclamide.

3.5. Hypoglycemic Effect of Sb Extract on Normal Wistar Rat Blood Sugar Level

Figure 4 shows the effect of the Sb extract (5 mg/kg bw and 25 mg/kg bw) and 0.9% NaCl on blood sugar levels in normal rats and diabetic rats induced by

alloxan.

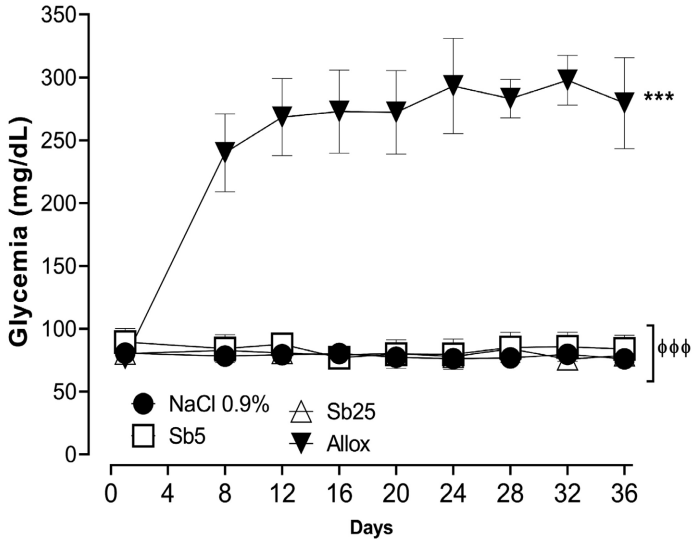

Figure 4. Hypoglycemic effect of the control NaCl 0.9% group, Alloxan group and the aqueous extract of *S. birrea* group on normal Wistar rat blood sugar level. Results are given as means ± SEM. where n = 6 rats per group. ***p < 0.001 versus Control NaCl 0.9% group and $^{\phi\phi\phi}$p < 0.001 vs Alloxan (Two-way ANOVA with Bonferroni's post hoc test was used). Abbreviation: Allox = Alloxan; Sb5: Decoction of *S. birrea* 5 mg/kg; Sb25: Decoction of *S. birrea* 25 mg/kg.

At inclusion, blood sugar levels were 80.67 ± 3.00 mg/dL, 89.50 ± 8.67 mg/dL, and 80.33 ± 5.00 mg/dL, respectively, for the animals in the control groups NaCl 0.9%, Sb5 and Sb25 without notable modification throughout the duration of the experiment. On the last day of the experiment, the values were 76.00 ± 3.67 mg/dL, 84.00 ± 9.00 mg/mL, and 78.83 ± 2.89 mg/dL, respectively, for the NaCl 0.9%, Sb5, and Sb25 groups. On the other hand, with 76.20 ± 3.76 mg/dL of blood glucose at inclusion, the animals in the Alloxan group presented a significant increase in blood sugar eight days after its administration (240.00 ± 22.40 mg/dL), which was maintained high until the 28th day of experimentation (279.60 ± 29.12 mg/dL).

3.6. Anti-Hyperglycemic Effect of Sb Aqueous Extract on Diabetic-Induced Wistar Rat Blood Sugar Level

The effects of Sb5 and Sb25 extracts and Glib on alloxan-induced hyperglycemia are shown in **Figure 5**.

Results show that all groups of rats had homogeneous blood sugar levels at baseline (80.67 ± 3.00 mg/dL, 76.20 ± 3.76 mg/dL, 84.83 ± 6.83 mg/dL, 84.33 ± 7.11 mg/dL and 80.67 ± 5.67 mg/dL for the control groups, Alloxan alone, All + Glib, All + Sb5 and All + Sb 25 respectively).

Eight days after the administration of Alloxan, the blood sugar levels of the different animals increased significantly with blood sugar values going from 84.83 ± 6.83 mg/dL to 84.33 ± 7.11 mg/dL and from 80.67 ± 5.67 mg/dL to 254.67 ±

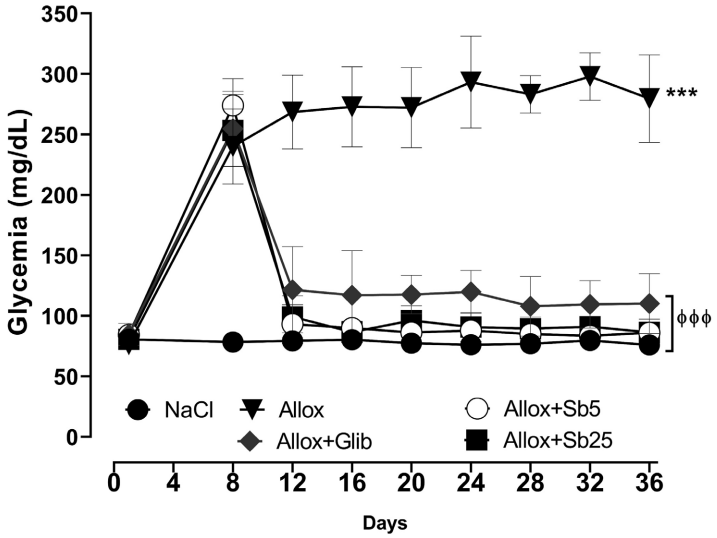

Figure 5. Anti-hyperglycemic effects of the aqueous decoction of *Sclerrocarya birrea* and glibenclamide on diabetic rats. Results are given as means ± SEM. where n = 6 rats per group. ***p < 0.001 versus Control NaCl 0.9% group and $^{\phi\phi\phi}$p < 0.001 vs Alloxan (Two-way ANOVA with Bonferroni's post hoc test was used). **Abbreviation**: Allox = Alloxan; Sb5: Decoction of *S. birrea* 5 mg/kg; Sb25: Decoction of *S. birrea* 25 mg/kg; Glib: Glibenclamide.

21.56 mg/dL, 273.83 ± 17.83 mg/dL and 253.17 ± 25.50 mg/dL respectively for the Allox + Glib, Allox + Sb5 and All groups + Sb25 compared to those of the control group (78.33 ± 6.11 mg/dL).

After four days of oral administration of Sb5 and Sb25, the blood sugar level of diabetic rats significantly decreased by more than 69%, going from 279.60 ± 36.25 to 86.00 ± 8.67 mg/dL for the Allox + Sb5 group and from 279.60 ± 36.25 mg/dL to 86.33 ± 10.98 mg/dL for the Allox + Sb25 group compared to the Allox alone group without ($p < 0.001$). In addition, glibenclamide (5 mg/kg/day) taken as a positive reference reduced the hyperglycemia of rats by 61%, going from 279.60 ± 36.25 mg/dL to 110.17 ± 24.70 mg/dL compared to the group Allox alone ($p < 0.001$) within the same period.

3.7. Effect of the Aqueous Decoction of *S. birrea* on the Average Rat's Body Weight Gain

Figure 6 shows the effects of Sb5 and Sb25 extracts (A) and Glib and Alloxan's impact on the animals' average weight gain during the experiment.

Analysis of Figure 6(a) shows that the extracts at daily doses of 5 and 25 mg/kg bw do not present any significant change in the evolution of the weight of the animals compared to that of the NaCl 0.9% control group. After 28 days of inclusion, the weight gain in the different groups was 30.00 ± 4.80 g (*i.e.*, 8.71% ± 1.59%), 20.50 ± 1.67 g (*i.e.*, 5.93% ± 0.27%), and 20.50 ± 2.33 g (*i.e.*, 5.94% ± 0.76%) compared to their respective initial weight of the NaCl 0.9%, Sb5 and Sb25 groups.

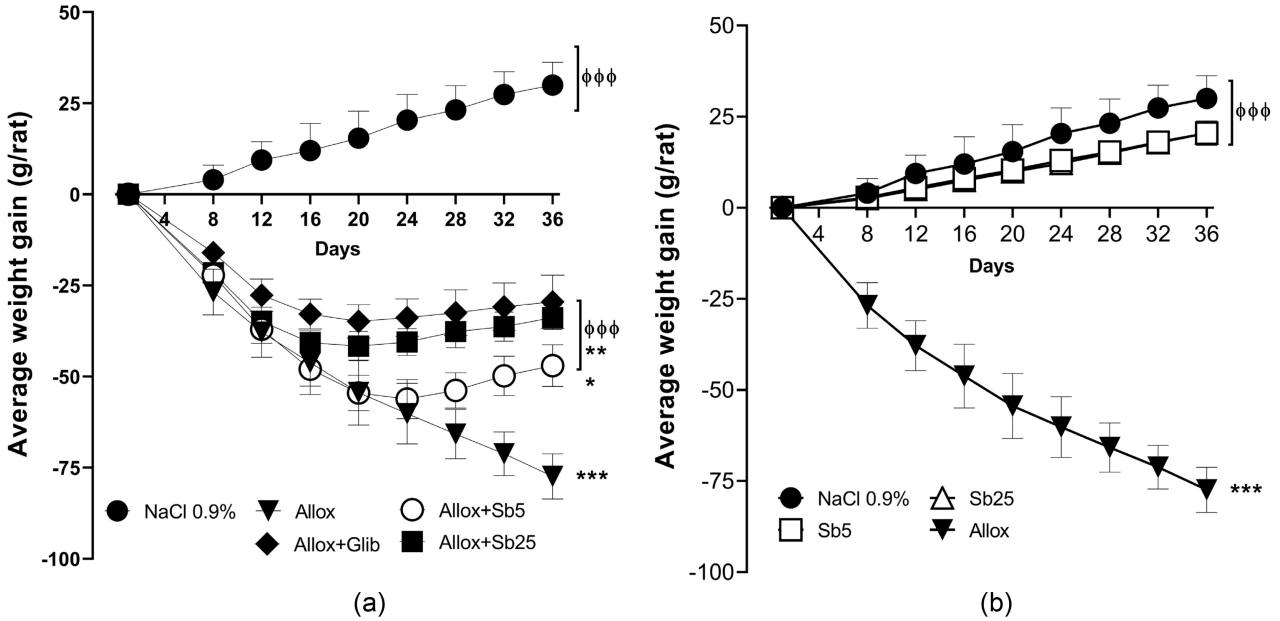

Figure 6. Effect of NaCl 0.9%, Sb5, Sb25, Glib and Allox (150 mg/kg once) on the mass gain of animals during experiment, (n = 6 rat per group). Values were expressed as Mean ± SEM; ***p < 0.001 versus Control NaCl 0.9% group and $^{\phi\phi\phi}$p < 0.001 vs Alloxan group. Abbreviation: Allox = Alloxan; Sb5: Decoction of *S. birrea* 5 mg/kg; Sb25: Decoction of *S. birrea* 25 mg/kg; Allox + Glib: Alloxan + Glibenclamide.

Results also show a weight loss of all diabetic groups from inclusion until day 12 with values of −37.8 ± 4.64 g (*i.e.* 10.02% ± 2.31%), −37.00 ± 2.67 g (*i.e.* 9.69% ± 1.19%), −34.83 ± 1.61 g (*i.e.* 9.37% ± 1.10%) and −27.67 ± 3.67 g (*i.e.* 7.55% ± 1.65%) respectively for the Alloxan alone, Allox + Sb5, Allox + Sb25, and Allox + Glib groups compared to their respective weight at inclusion (**Figure 6(b)**). A slight but significant weight gain was then gradually observed for the Allox + Glib and Allox + Sb25 groups compared to the Alloxan alone and Allox + Sb5 groups until the end of the experiment. On day 28, the respective weight values of the different groups were −77.40 ± 4.88 g (*i.e.* 21.86% ± 1.73%), −47.00 ± 4.67 g (*i.e.* 13.30% ± 1.05%), −33.83 ± 2.50 g (*i.e.* 9.91 ± 0.75%) and −29.50 ± 5.17 g (*i.e.* 8.64% ± 1.51%) for the group's Alloxan alone, Allox + Sb5, Allox + Sb25, and Allox + Glib.

3.8. Effect of the Lyophilized Aqueous Extract of *S. birrea* on Animal's Average Water Consumption

Table 4 shows the effects of Sb5 and Sb25 extracts, Glib and Alloxan, on the water consumption of treated animals during the experiment.

On the one hand, results show that the water consumption of the animals in the groups showed no statistically significant difference in the Sb5 and Sb25 groups compared to the NaCl 0.9% control group throughout the experiment.

On the other hand, a very intense and significant consumption of the animals made diabetic by alloxan alone was maintained until the end of the experiment ranging from 170.24 ± 36.39 mL/Rat (D8) to 220.83 ± 00 mL/Rat (D28) compared

to the NaCl 0.9% groups [64.29 ± 2.04 mL/Rat (D8) and 94.79 ± 1.56 (D28)]. Moreover, the diabetic animals from the Allox + Glib (187.5 ± 12.5 mL/Rat, D12), Allox + Sb5 (189.58 ± 6.25 mL/Rat, D12) and Allox + Sb25 (187.5 ± 4.17 mL/Rat, D12) groups had presented a progressive and significant reduction in their water consumption during treatment to reach respective values at D28 of 114.58 ± 2.08 mL/Rat (Allox + Glib), 117.70 ± 3.65 mL/Rat (Allox + Sb5) and 108.33 ± 00 mL/Rat (Allox + Sb25) compared to the Alloxan groups alone and NaCl 0.9% (Table 4). Exceptionally, no difference was observed on D28 between the Allox + Sb25 group compared to the NaCl 0.9% control group.

Table 4. Effect of NaCl 0.9%, Sb5, Sb25, Glib and Allox (150 mg/kg once) on the average of animals water consumption during experiment, (n = 6).

Average Water consumption during the various treatments (mL/Rat)							
Days	**NaCl 0.9%**	**Sb5**	**Sb25**	**Allox**	**Allox + Glib**	**Allox + Sb5**	**Allox + Sb25**
8	64.29 ± 2.04$^{\phi\phi\phi}$	63.69 ± 1.70$^{\phi\phi\phi}$	67.86 ± 1.70$^{\phi\phi\phi}$	170.24 ± 36.39***	164.29 ± 40.81***	176.19 ± 43.53***	179.16 ± 41.70***
12	68.75 ± 2.08$^{\phi\phi\phi}$	66.67 ± 1.56$^{\phi\phi\phi}$	71.87 ± 1.56$^{\phi\phi\phi}$	204.17 ± 4.17***	187.50 ± 12.50***	189.58 ± 6.25***	187.50 ± 4.17***
16	70.83 ± 0.00$^{\phi\phi\phi}$	70.83 ± 0.00$^{\phi\phi\phi}$	75.02 ± 0.00$^{\phi\phi\phi}$	208.33 ± 0.00***	170.83 ± 2.08***$^{\phi\phi\phi}$	181.25 ± 3.12***$^{\phi\phi\phi}$	170.83 ± 6.25***$^{\phi\phi\phi}$
20	75.00 ± 0.00$^{\phi\phi\phi}$	71.87 ± 1.56$^{\phi\phi\phi}$	79.17 ± 2.08$^{\phi\phi\phi}$	212.50 ± 4.17***	150.00 ± 12.50***$^{\phi\phi\phi}$	166.67 ± 4.17***$^{\phi\phi\phi}$	145.83 ± 4.17***$^{\phi\phi\phi}$
24	79.17 ± 2.08$^{\phi\phi\phi}$	76.04 ± 1.56$^{\phi\phi\phi}$	79.17 ± 2.08$^{\phi\phi\phi}$	219.79 ± 1.56***	130.21 ± 1.56***$^{\phi\phi\phi}$	154.17 ± 6.25***$^{\phi\phi\phi}$	137.50 ± 2.08***$^{\phi\phi\phi}$
28	87.50 ± 0.00$^{\phi\phi\phi}$	81.21 ± 2.08$^{\phi\phi\phi}$	82.29 ± 1.56$^{\phi\phi\phi}$	220.83 ± 0.00***	128.13 ± 1.56***$^{\phi\phi\phi}$	137.5 ± 4.17***$^{\phi\phi\phi}$	127.08 ± 2.08***$^{\phi\phi\phi}$
32	90.62 ± 1.56$^{\phi\phi\phi}$	84.37 ± 1.56$^{\phi\phi\phi}$	84.38 ± 1.56$^{\phi\phi\phi}$	220.83 ± 0.00***	122.92 ± 3.13***$^{\phi\phi\phi}$	127.08 ± 2.08***$^{\phi\phi\phi}$	114.58 ± 2.08**$^{\phi\phi\phi}$
36	94.79 ± 1.56$^{\phi\phi\phi}$	89.58 ± 2.08$^{\phi\phi\phi}$	87.5 ± 0.00$^{\phi\phi\phi}$	220.83 ± 0.00***	114.58 ± 2.08*$^{\phi\phi\phi}$	117.70 ± 3.65**$^{\phi\phi\phi}$	108.33 ± 0.00$^{\phi\phi\phi}$

Note: Values were expressed as Mean ± SEM; *p < 0.05, **p < 0.01 and ***p < 0.001 versus Control NaCl 0.9% group; $^{\phi\phi\phi}$p < 0.001 vs Alloxan group. *Abbreviation*: Allox = Alloxan; Sb5: Decoction of *S. birrea* 5 mg/kg; Sb25: Decoction of *S. birrea* 25 mg/kg; Glib: Glibenclamide.

3.9. Effect of the Lyophilized Aqueous Extract of *S. birrea* on the Average Food Consumption of Treated Animals

Table 5 shows the effects of Sb5 and Sb25 extracts, Glib and Alloxan on the average food consumption of treated animals during the experiment are presented in Table 5.

Analysis of Table 5 shows that the average consumption of the animals was identical at inclusion (data not shown), but changes were observed depending on the treatments administered during the experiment.

No difference was observed in the average food consumption in the animals receiving Sb5 and Sb25 compared to that of the 0.9% NaCl control group throughout the experiment (D4 to D36). Their average consumption increased from 18.93 ± 0.99 g/Rat, 18.11 ± 0.86 g/Rat and 19.25 ± 0.56 g/Rat (D4) to 22.07 ± 1.20 g/Rat, 21.94 ± 1.65 g/Rat and 23.00 ± 0.70 g/Rat respectively for the animals in the groups treated with Sb5, Sb25 and NaCl 0.9% (D36).

Table 5. Effect of NaCl 0.9%, Sb5, Sb25, Glib, and Allox (150 mg/kg once) on the average of animals' food consumption during the experiment (n = 6).

	Average Feed consumption during the various treatments (g/Rat)						
Days	**NaCl 0.9%**	**Sb5**	**Sb25**	**Allox**	**Allox + Glib**	**Allox + Sb5**	**Allox + Sb25**
J4	19.25 ± 0.56$^{\phi\phi\phi}$	18.93 ± 0.99$^{\phi\phi\phi}$	18.11 ± 0.86$^{\phi\phi\phi}$	26.64 ± 0.25***	25.90 ± 0.78***	27.28 ± 1.19***	27.54 ± 1.08***
J8	19.88 ± 0.65$^{\phi\phi\phi}$	19.51 ± 1.04$^{\phi\phi\phi}$	18.70 ± 0.88$^{\phi\phi\phi}$	35.25 ± 1.24***	35.71 ± 1.23***	37.65 ± 1.53***	35.76 ± 1.44***
J12	20.13 ± 0.61$^{\phi\phi\phi}$	19.69 ± 1.04$^{\phi\phi\phi}$	19.05 ± 0.89$^{\phi\phi\phi}$	35.13 ± 0.77***	33.10 ± 3.00***	32.36 ± 1.38***	33.35 ± 1.42***
J16	20.58 ± 0.70$^{\phi\phi\phi}$	20.21 ± 1.09$^{\phi\phi\phi}$	19.40 ± 0.90$^{\phi\phi\phi}$	35.15 ± 0.91***	27.12 ± 1.10$^{\phi\phi\phi}$***	27.90 ± 1.24$^{\phi\phi\phi}$***	30.22 ± 1.18$^{\phi\phi\phi}$***
J20	20.96 ± 0.70$^{\phi\phi\phi}$	20.46 ± 1.12$^{\phi\phi\phi}$	19.70 ± 0.91$^{\phi\phi\phi}$	34.29 ± 1.09***	24.66 ± 0.96$^{\phi\phi\phi}$***	24.21 ± 1.04$^{\phi\phi\phi}$***	26.76 ± 0.95$^{\phi\phi\phi}$***
J24	21.46 ± 0.66$^{\phi\phi\phi}$	20.89 ± 1.14$^{\phi\phi\phi}$	19.88 ± 0.93$^{\phi\phi\phi}$	33.75 ± 1.02***	22.51 ± 0.87$^{\phi\phi\phi}$	21.98 ± 0.92$^{\phi\phi\phi}$	21.44 ± 0.81$^{\phi\phi\phi}$
J28	21.92 ± 0.70$^{\phi\phi\phi}$	21.38 ± 1.20$^{\phi\phi\phi}$	20.40 ± 0.92$^{\phi\phi\phi}$	33.40 ± 0.88***	21.94 ± 0.86$^{\phi\phi\phi}$	21.23 ± 0.90$^{\phi\phi\phi}$	21.08 ± 0.79$^{\phi\phi\phi}$
J32	22.46 ± 0.71$^{\phi\phi\phi}$	21.75 ± 1.19$^{\phi\phi\phi}$	21.95 ± 1.63$^{\phi\phi\phi}$	33.58 ± 0.82***	21.54 ± 0.81$^{\phi\phi\phi}$	20.88 ± 0.92$^{\phi\phi\phi}$	21.68 ± 1.72$^{\phi\phi\phi}$
J36	23.00 ± 0.70$^{\phi\phi\phi}$	22.07 ± 1.20$^{\phi\phi\phi}$	21.94 ± 1.65$^{\phi\phi\phi}$	32.81 ± 0.85***	22.18 ± 1.53$^{\phi\phi\phi}$	22.06 ± 1.68$^{\phi\phi\phi}$	21.59 ± 1.86$^{\phi\phi\phi}$

Note: Values were expressed as Mean ± SEM; ***p < 0.001 versus Control NaCl 0.9% group; $^{\phi\phi\phi}$p < 0.001 vs Alloxan group. *Abbreviation*: Allox = Alloxan; Sb5: Decoction of *S. birrea* 5 mg/kg; Sb25: Decoction of *S. birrea* 25 mg/kg; Glib: Glibenclamide.

A significant increase in average food consumption was observed in Alloxan-induced diabetic animals (Allox group) from D4 (26.64 ± 0.25 g/Rat) to D36 (32.81 ± 0.85 g /Rat) compared to that of the NaCl 0.9% control group. Such a significant difference in average food consumption was also observed in the Allox + Glib group [(25.90 ± 0.78 g/Rat, D4) and (24.66 ± 0.96 g/Rat, D20)], in the Allox + Sb5 group [(27.28 ± 1.19 g/Rat, D4) and (24.21 ± 1.04 g/Rat, D20)] and in the Allox + Sb25 group [(27.54 ± 1.08 g/Rat, D4) and (26.76 ± 0.95 g/Rat, D20)] from D4 to D20 compared to that of the NaCl 0.9% control group. On the other hand, no difference in food consumption in the treated animals of the Allox + Glib, Allox + Sb5, and Allox + Sb25 groups was observed from D24 until the end of the experiment (D36) compared to that of the control group NaCl 0.9% (Table 5).

3.10. Effect of the Lyophilized Aqueous Extract of *S. birrea* on Average Organ Weight after Necropsy

The effects of Sb5, Sb25, Allox, and Glib on the average weight of the organs after the animal's necropsy is presented in Table 6.

Analysis of the results shows that the different doses of extracts administered alone (Sb5 and Sb25) did not significantly differ in the average weight of the six (06) organs taken compared to the NaCl 0.9% control group. A very significant increase in the average weight of the different organs collected and weighed for diabetic animals (Allox) compared to those of the control group except for the spleen, where the difference is significant (Allox = 0.27 ± 0.02 g) compared to the Sb5 (0.21 ± 0.02 g), Sb25 (0.22 ± 0.02 g) and control (0.20 ± 0.03 g) groups respectively.

Treatment with the extracts (Sb5 and Sb25) and glibenclamide in diabetic animals shows no statistically significant difference in the increase in the weight

Table 6. Average weight of the organs after the animal necropsy.

Organs	NaCl 0.9%	Sb5	Sb25	Allox	Allox + Glib	Allox + Sb5	Allox + Sb25
Heart (g/g)	0.33 ± 0.01$^{\phi\phi\phi}$	0.35 ± 0.03$^{\phi\phi\phi}$	0.34 ± 0.03$^{\phi\phi\phi}$	0.44 ± 0.04***	0.39 ± 0.03	0.40 ± 0.03	0.39 ± 0.03
Liver (g/g)	2.71 ± 0.16$^{\phi\phi\phi}$	2.84 ± 0.15$^{\phi\phi}$	2.68 ± 0.17$^{\phi\phi\phi}$	3.59 ± 0.23***	3.28 ± 0.18*	3.34 ± 0.32*	3.32 ± 0.29*
Lungs (g/g)	0.49 ± 0.03$^{\phi\phi\phi}$	0.54 ± 0.04$^{\phi\phi\phi}$	0.49 ± 0.05$^{\phi\phi\phi}$	0.72 ± 0.04***	0.56 ± 0.02$^{\phi\phi\phi}$	0.63 ± 0.06**	0.62 ± 0.04**
Spleen (g/g)	0.20 ± 0.03$^{\phi\phi}$	0.21 ± 0.02$^{\phi}$	0.22 ± 0.02$^{\phi}$	0.27 ± 0.02**	0.23 ± 0.02	0.23 ± 0.02	0.24 ± 0.03
Kidneys (g/g)	0.65 ± 0.02$^{\phi\phi\phi}$	0.68 ± 0.05$^{\phi\phi\phi}$	0.66 ± 0.05$^{\phi\phi\phi}$	0.90 ± 0.08***	0.79 ± 0.06*	0.79 ± 0.04**	0.79 ± 0.03*

Note: Values were expressed as Mean ± SEM; *p < 0.05, **p < 0.01 and ***p < 0.001 versus Control NaCl 0.9% group; $^{\phi\phi\phi}$p < 0.001 vs Alloxan group. *Abbreviation*: Allox = Alloxan; Sb5: Decoction of *S. birrea* 5 mg/kg; Sb25: Decoction of *S. birrea* 25 mg/kg; Glib: Glibenclamide.

of the heart, spleen, and testes of the initially diabetic animals compared to those of the control group. Treatment with extracts (Sb5 and Sb25) reduced the increase in liver weight (Sb = 3.34 ± 0.32 g and Sb25 = 3.32 ± 0.29 g) and that of the kidneys. (0.79 ± 0.04 g and Sb25 = 0.79 ± 0.03 g) but without statistically significant difference compared to the NaCl 0.9% control group (2.71 ± 0.16 g for the liver and 0.65 ± 0.02 g for the kidneys). However, glibenclamide significantly reduced the increase in the average lung weight of the animals (0.56 ± 0.02 g) compared to those in the Allox group (Table 6).

3.11. Effect of the Lyophilized Aqueous Extract of *S. birrea* on Treated Rats'

Table 7 presents the effects of Sb5, Sb25, Allox, and Glib on the animals' biochemical parameters.

Table 7. Effect of the lyophilized aqueous extract of *S. birrea* on treated rat liver parameters.

Biochemical parameters	NaCl 0.9%	Sb5	Sb25	Allox	Allox + Glib	Allox + Sb5	Allox + Sb25
TC (mmol/L)	1.08 ± 0.05	1.33 ± 0.10*	1.11 ± 0.07	1.49 ± 0.16***	1.21 ± 0.08$^{\#\#}$	1.81 ± 0.12***$^{\#\#\$\$\$}$	1.02 ± 0.10$^{\#\#\#\$}$
HDL (mmol/L)	0.37 ± 0.05	0.47 ± 0.05	0.46 ± 0.04	0.48 ± 0.07	0.49 ± 0.05	0.59 ± 0.04*	0.50 ± 0.05
TG (mmol/L)	0.23 ± 0.03	0.28 ± 0.11	0.17 ± 0.05	0.32 ± 0.07	0.34 ± 0.05	0.27 ± 0.02	0.26 ± 0.05
LDL (mmol/L)	0.73 ± 0.20	0.87 ± 0.23	0.60 ± 0.12	0.66 ± 0.20	0.58 ± 0.11	1.09 ± 0.15***$^{\#\#\#\$\$\$}$	0.39 ± 0.10***$^{\#\#\$}$

Note: Values were expressed as Mean ± SEM; *p < 0.05, and ***p < 0.001 versus Control NaCl 0.9% group; $^{\#}$p < 0.05, $^{\#\#}$p < 0.01 and $^{\#\#\#}$p < 0.001 versus Allox group; $^{\$}$p < 0.05, $^{\$\$\$}$p < 0.001 vs Allox + Glib group. *Abbreviation*: Allox = Alloxan; Sb5: Decoction of *S. birrea* 5 mg/kg; Sb25: Decoction of *S. birrea* 25 mg/kg; Glib: Glibenclamide.

Analysis of Table 7 shows that the daily dose of 5 mg/kg of *S. birrea* (Sb5) causes a slight but significant increase in TC in normal animals (Sb5 = 1.33 ± 0.10 mmol/L) as well as in alloxan-induced diabetics rats (Allox+ Sb5 = 1.81 ± 0.12 mmol/L) compared to those of the normal control group (NaCl 0.9% = 1.08 ± 0.05 mmol/L). Glibenclamide taken as a positive control as well as Sb25 led to a

significant reduction in TC compared to the Alloxan treatment alone (1.49 ± 0.16 mmol/L) with values of 1.21 ± 0.08 mmol/L and 1.02 ± 0.10 mmol/L respectively for the Allox + Glib and Allox + Sb25 groups.

Also, the different treatments led to a non-significant increase in the HDL level in all the study groups with the exception of the Allox + Sb5 group (0.59 ± 0.04 mmol/L) compared to the NaCl 0.9% control group (0.37 ± 0.05 mmol/L).

A slight but non-significant variation in TG was observed for all the intervention groups compared to those of the normal control group. These TG values were of 0.28 ± 0.11 mmol/L, of 0.17 ± 0.05 mmol/L, of 0.32 ± 0.07 mmol/L, of 0.34 ± 0.05 mmol/L, of 0.27 ± 0.02 mmol/L and of 0.26 ± 0.05 respectively for the treatment with Sb5, Sb25, Allox, Allox + Sb5, Allox + Sb25 and 0.23 ± 0.03 mmol/L for the NaCl 0.9% group.

Moreover, the treatment with Sb5 led to a significant increase in the level of LDL in diabetic animals (Allox + Sb5 = 1.09 ± 0.15 mmol/L) and not significant in non-diabetics (Sb25 = 0.87 ± 0.23 mmol/L) compared to that of the control group. On the other hand, Sb25 led to a very significant drop in the LDL level in diabetic animals (Allox + Sb25 = 0.39 ± 0.10 mmol/L) compared to that of the control group (NaCl 0.9% = 0.73 ± 0.20 mmol/L) and which is more marked than that of the reference used (Allox + Glib = 0.58 ± 0.11 mmol/L).

4. Discussion

Diabetes is a serious pathology of a multifactorial nature, responsible for deleterious impacts on health and whose therapeutic regimen is very complex in modern medicine [18]-[20]. Current therapy mainly targets α-glucosidase inhibitors, SGLT-2 cotransporter inhibitors, DPP-IV and pancreatic GLP-1 receptor inhibitors, metformin, meglitinide analogs, and sulfonylureas [21]-[23]. Unfortunately, multiple side effects related to these drugs have sparked a growing interest among populations, particularly Africans, for products derived from traditional medicine against this pathology [24] [25]. The present study aimed to characterize the phytochemistry and evaluate, in vivo, the antidiabetic effects of the lyophilized aqueous decoction of *S. birrea*, a plant commonly used in traditional medicine in Burkina Faso against diabetic pathology known to be a disease that is difficult to identify with a strong harmful impact on patients. To verify whether the lyophilized aqueous decoction of *S. birrea* had a potential antidiabetic effect, the experiment consisted of determining the hypoglycemic and anti-hyperglycemic effects in subacute administration over 28 days of this extract at doses of 5 mg/kg and 25 mg/kg of body weight. The impact of these doses on the weight, food, and water consumption, as well as the lipid parameters of the treated animals, was determined. During the study, results showed that none of the doses of Sb (5 mg/kg or 25 mg/kg) used alone on normoglycemic animals caused lethality during the entire experimental period. These results are comparable to those of the Allox + Glib group, suggesting a possible safe use of this extract. These findings corroborate our previous studies, which showed that freeze-dried aqueous decoction had an

estimated lethal dose of 5000 mg/kg and was considered to be of low toxicity [26]. These results align with recent studies showing that extracts of *S. birrea* leaves and trunk bark did not cause animal mortality [27] [28]. However, a loss of two rats out of six was recorded in the Allox-only group, 7 days after induction of diabetes, confirming the toxicity of Alloxan and the difficulty of this experimental model. This mortality is in line with the literature, according to which alloxan, a toxic glucose analog, is a selective hyper-toxic diabetogen of pancreatic insulin-secreting cells. Alloxan thus creates mitochondrial dysfunction, responsible for mortality in experimental animals [29] [30]. Indeed, alloxan causes, among other things, hypo-insulinemia and hyperglycemia phenomena with an accumulation of free radicals that are responsible for significant toxicity in animals. No mortality was observed after Sb treatment (5 mg/kg and 25 mg/kg) was combined with alloxan in diabetic rats. This observation could be explained by the fact that Sb treatment has the capacity to neutralize, attenuate, or inhibit the cytotoxic effects of alloxan at the mitochondrial level. Indeed, our previous findings have shown that this extract is rich in phenolic and flavonoid compounds [27]. The same study also reported a powerful antioxidant effect, notably an anti-free radical. Thus, this Sb extract can reduce or even inhibit the accumulation of alloxan-induced free radicals in pancreatic cells.

The research on the hypoglycemic effect of Sb5 and Sb25 was then conducted in normoglycemic rats and aimed to determine their potential action to induce hypoglycemia, a real source of side effects often criticized for modern drugs in diabetics. The results showed that the different extracts administered alone did not cause any notable variation in the blood sugar of normoglycemic animals, which remained comparable to that of the 0.9% NaCl control group. In this study, hypoglycemia was declared when a blood sugar level below 70 mg/dL was recorded in rats. It becomes severe and then moderate for values of 40 mg/dL and 60 mg/dL respectively [31] [32]. Thus, the present results suggest that even if the doses Sb5 and Sb25 were used inadvertently, they would not present a major hypoglycemic risk. It is also known that the hypoglycemic activity of many plant species was linked to the presence of phytochemical compounds such as tannins, phenols, saponins, or flavonoids [33]. Despite the presence of these chemical compounds in the Sb extract, safety of use can be considered in the range of doses tested. To evaluate the antidiabetic effects of the plant extract, a single dose of alloxan at 150 mg/kg body weight was administered to rats in the Allox, Allox + Sb5, Allox + Sb25, and Allox + Glib groups, followed by treatment with the extracts after the onset of diabetes. The present study showed that daily administration of Sb5, Sb25 extracts, and glibenclamide in alloxan-induced diabetic rats significantly reduced their fasting blood glucose levels with a return to normal values after only 4 days of treatment. This result suggests that the doses of extract tested induced a certain antidiabetic effect by opposing the accumulation of blood glucose with a reduction in the level available in the blood. This could be explained by an inhibition of hepatic glycogenolysis and, to some extent, gluconeogenesis

since studies have shown that stimulation of glycogenolysis is a source of blood hyperglycemia via increased accumulation of glucose in the blood [34] [35]. In addition, the antidiabetic effects of Sb5 and Sb25 could be explained by a possible improvement in the activity of pancreatic beta cells with an "insulin"-like effect similar to metformin in diabetics [34] [35]. Thus, the extracts could act on each glucose cellular signaling pathway at the pancreatic beta cell level, such as blocking the direct release of glucose or closing ATP-dependent potassium channels, reducing intracellular calcium levels, inhibiting the exocytosis mechanism, etc. [36]. This hypothesis could be supported because this extract contains chemical compounds capable of modulating signaling pathways leading to glucose release or glycogen biosynthesis. Studies have shown that ellagic acid, kaempferol, and their derivatives can reduce oxidative stress in diabetic patients. [37]. Thus, phytochemical compounds such as vanillic-4-sulfate and rhamnetin found in the Sb extract by LC-MS analysis would contribute enormously to diabetes management. This could explain the return to baseline blood sugar levels in diabetic rats treated with the extract.

Similarly, it is well known that alloxan accelerates the production of free radicals responsible for liver cell toxicity, generating a cascade of reactions and an increase in blood glucose [38]. The antidiabetic effect of Sb5 and Sb25 could be due to inhibiting the production of these free radicals since a recent study has already shown that these doses of the lyophilized aqueous extract of *S. birrea* were endowed with antioxidant properties. Other authors have also shown that various extracts of various parts of this plant exhibit antioxidant activities [39] [40]. More interestingly, the doses of Sb5 and Sb25 induced. Still, without significant difference, an antidiabetic effect superior to that of glibenclamide used as a reference control, suggesting that the extract could, in the future, constitute a candidate for developing an antidiabetic candidate. Above all, the present study showed that in daily administration, Sb5 and Sb25 do not induce any negative impact on the evolution of the weight of the animals during the 28 days of treatment, which remained comparable to that of the animals in the NaCl 0.9% Control group. These results suggest that these doses of *S. birrea* extract do not have any potential anorexic effects, hyperhydration, or overhydration (hyponatremia). On the other hand, alloxan administered alone resulted in a sudden and very significant loss of body weight in animals throughout the experiment compared to those in the 0.9% NaCl control group. This result could be explained by the toxicity of alloxan on hepatic cells, leading to a reduction in the production of the growth hormone insulin [41] [42]. Indeed, weight loss is an intuitive feature of the onset of diabetes, and many studies have shown that the action of alloxan is correlated with significant weight loss in animals that have been made diabetic by this sulfonylurea [43] [44]. On the other hand, in diabetic animals induced by alloxan, the daily administration of Sb5, Sb25, or glibenclamide led to a progressive weight gain in the animals from the 12th day of treatment, and this until the end of the experiment but without regaining their initial weight. These results agree with those of other

authors who have shown that many plant extracts with antidiabetic properties improved the weight loss of animals [44] [45]. In addition, the present study revealed a significant increase in the weight of the heart, liver, spleen, lungs, kidneys, and testes in diabetic animals following treatment compared to those in the control group. These results are in agreement with those of previous studies that have shown such an incidence of alloxan in experimental diabetic animals with tissue damage of these organs [37]. On the other hand, treatment with Sb5 or Sb25 extracts did not impact the weight of these organs compared to those of the 0.9% NaCl control group, indicating that the tested doses did not cause tissue damage in normal rats. Also, it emerges from this study that in diabetic animals induced by alloxan, Sb5 and Sb25 extracts caused a slight but non-significant reduction in the weight of the liver, lungs, and kidneys compared to the Allox group alone. In addition, this study showed that the macroscopic analysis of their appearance did not experience any specific change. Still, histopathological analyses are necessary to properly judge the possible impact of the different treatments on these target organs. These different results would, therefore, be of great scientific interest in the event of valorization of this extract. In addition, it is known that the onset of type 2 diabetes is associated with a drastic increase in triglyceride levels and low-density lipoprotein cholesterol (LDL-c) but with a decrease in high-density lipoprotein cholesterol (HDL-c) levels responsible for atherosclerosis in the vessels [46] [47]. In the present study, the results showed a significant increase in the rate of TC but not significant in the TG and LDL of animals in the Alloxan group compared to those in the 0.9% NaCl control group. These results agree with those of other authors who have shown an increase in these lipid parameters in diabetic animals induced by alloxan [48] [49]. The difference could be attributable to a possible self-recovery in alloxan-induced diabetic animals with a dose of 150 mg/kg bw based on the hypotheses of other authors [50]-[53]. Also, referring to these lipid parameters (CT, TG, and LDL-c), it would be appropriate for the Sb25 dose to be preferred in the event of the development of a phytomedicine candidate since Sb5 is nevertheless responsible for a slight increase in these parameters during this experiment. However, further investigations must determine their direct impact on liver cells with these doses. Interestingly, it emerges from the present study that the amount of HDL increased slightly in the different groups treated with Sb5, Sb25 (alone or with Allox) and Glibenclamide. However, this was not significant compared to those of the NaCl 0.9 control group and the Allox group. Indeed, the increase in HDL levels facilitates insulin secretion by pancreatic beta cells and a modification of glucose uptake by somatic muscles but is also responsible for important anti-inflammatory and antioxidant activities [54].

The presence of chemical compounds such as flavonoids, tannins, and saponosides in the decoction of *S. birrea* trunk bark, reported in a recent study, could account for the pharmacological properties observed [25]. In addition, the HPLC-MS analysis report showed the presence of vanillic acid 4-sulfate and rhamnetin. These results corroborate those of other authors who have also highlighted the

presence of these compounds in *S. birrea* stem and leaf bark extracts [55] [56]. Better still, Alqudah *et al.* have demonstrated that isorhamnetin, an isomer of rhamnetin, can reduce glycemia by activating the PGK1/AKT pathway [57] [58]. Previous studies have also shown that vanillic acid and its derivatives protect against diabetes [59] [60]. Although the phytochemical study was unable to isolate and quantify these molecules of interest, detecting these compounds in the Sb extract must be recognized as valuable information in discovering natural molecules with antidiabetic activity. Further studies are therefore underway to better elucidate these molecules' origin and synthesis mechanics.

5. Conclusion

The present work demonstrated the antidiabetic properties of *S. birrea* trunk bark decoction on rats made diabetic with alloxan. In addition, this work reported a non-hypoglycemic effect of the extract on healthy non-diabetic rats. Phytochemical LC-MS analysis detected the presence of vanillic acid 4-sulfate, rhamnetin, and other compounds of interest in the extract concerned. Taken together, these findings could help demonstrate the antidiabetic efficacy of this extract. All of which may contribute to justifying its use in traditional medicine to manage diabetes. Further work will be carried out to elucidate this drug candidate's molecular and cellular mechanisms of action.

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Conflicts of Interest

The authors declare that there are no conflicts of interest in the publication of this article.

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