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Effect of aqueous extract of Scorodophloeuszenkeri bark on chronic hyperglycemia and its complications in a diabetic Wistar rat model induced by streptozotocin

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ABSTRACT

Diabetes and its complications represent a real major public health problem in the world because of its high rates of morbidity and mortality. Chronic hyperglycemia, oxidative stress, dyslipidemia and inflammation play a major role in the pathophysiology of diabetes and its vascular complications. The objective of this study was to evaluate the effect of aqueous extract of S. zenkeri on chronic hyperglycemia and its complications in a streptozotocin-induced diabetic Wistar rat model. The barks of S. zenkeri were washed, dried and crushed; the powder was dissolved in distilled water (1:10 weight/volume) then macerated and the filtrate obtained was dried in an oven. Subsequently, after quantification of the bioactive compounds (total polyphenols, flavonoids and alkaloids) present in the extract, an in vivo study was conducted in an animal model of streptozotocin-induced hyperglycemia. For this fact, the rats were divided into four groups of five rats as folow: a normoglycaemia group (NC), an untreated hyperglycaemia group (PC), two hyperglycaemia groups including a test group receiving by esophageal gavage, the aqueous extract of the bark of S zenkerii (AESZ) at a dose of 300 mg/kg body weight and a control group receiving metformin at a dose of 20 mg/kg body weight. During the treatment which lasted 21 days, the weights have been taken every two days and the blood sugar levels every week. At the end of the treatment, the rats were killed under light chloroform anesthesia; the plasma, hemolysate, serum and liver homogenate prepared were used to assay the biochemical parameters of oxidative stress (catalase, MDA), lipid profile (Triglycerides, total cholesterol and HDL-cholesterol) and immunological (CRP and NFS). It emerged that the extract limited weight loss and caused a reduction in blood sugar of −26.59% after 21 days of treatment; the extract caused an increase in the activity of erythrocyte catalase and a reduction in the concentration of hepatic MDA, as well as a very marked reduction in inflammatory cells and CRP. The extract also caused a reduction in dyslipidemiawhich was materialized by a reduction in CRR, AC, AIP and an increase in CPI. These results suggest that this extract contains bioactive compounds capable of reducing chronic hyperglycemia while preventing its complications, thus justifying its traditional use in the management of diabetes.

1. Introduction

Diabetes is currently one of the main pathologies among the diseases of civilization due to its high rates of morbidity and mortality [1]. It can be defined as a metabolic disorder characterized by chronic hyperglycemia due to impaired carbohydrate, lipid and protein metabolism [2]. According to the International Diabetes Federation (IDF), more than 463 million people have diabetes worldwide (IDF, 2019). Cameroon is not spared because it has 615,300 people with diabetes (FID, 2020). It's a pathology traditionally classified into two types; however other types can be added to meet specific situations (Nabarun et *al.*, 2019). Type I diabetes known as insulin-dependent diabetes is characterized by deficient insulin production [3]. Type II commonly called non-insulin dependent diabetes, characterized by impaired insulin secretion and abnormal effects of insulin on its target tissues (insulin sensitivity) [4]. Diabetes of any type leads to several long-term complications, including

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microvascular (retinopathy, neuropathy, nephropathy) and macrovascular complications [5]. Indeed, hyperglycemia induces oxidative stress, thus generating an increased production of reactive oxygen/-nitrogen species (ROS/RNS) responsible for suppressing antioxidant defenses (superoxide dismutase, catalase, glutathione peroxidase) and alteration of epigenetic mechanisms. In addition, oxidative stress induced by hyperglycemia contributes to the abnormal activation of pro-inflammatory signaling pathways, including the activation of the transcription factor NF-κβ, leading to chronic inflammation [6]. It is known that inflammatory mediators are potential massive attractors of white blood cells responsible for the vascular lesions observed in the onset and development of vascular complications of diabetes [7].

Faced with this pathology, management can be done using a non-pharmacological approach based on the consumption of diets rich in fiber and low in fat [8]. But also, through physical exercise improving insulin sensitivity in the muscles [9]. When lifestyle changes that are difficult to implement and maintain are not sufficient to control glycemia, it is then important to resort to pharmacological approach based on the use of oral antidiabetics, antioxidants and anti-inflammatories [10, 11]. To date, all these therapeutic approaches have not limited the high mortality associated with this pathology. In addition, these drugs in combination or non-combination therapies are an important source of xenobiotics with harmful effects in the body and are associated with other disadvantages such as drug resistance [12]. To this end, research is increasingly focused on the use of less toxic and less expensive natural plants as a means of managing diabetes and its complications [13].

Plants are an important source of substances with therapeutic potential such as polyphenols, flavonoids and alkaloids [14]. These bioactive compounds confer to plants a potential biological activity such as anti-inflammatory, antioxidant, hepatoprotective, hypolipidemic and hypoglycemic [14–16]. Scorodophloeuszenkeri is a plant of the Fabaceae family; commonly called garlic tree. Because of their contribution in flavor (characteristic garlic flavor) and aroma, its barks and fruits are used mainly to season food. In Cameroon they are used as spices especially in the preparation of traditional sauces Nah poh (yellow sauce) and Nkui (sticky sauce) [17]. The studies conducted by Etoundi et al. [18] showed respectively that the fruits, barks and leaves of Scorodophloeuszenkeri are rich in bioactive compounds of interest (polyphenols, flavonoids and alkaloids). The presence of these bioactive compounds gives this plant antioxidant, antihyperlipidemic and antihyperglycemic activity [18,19]. The objective of this study was to evaluate the ability of bioactive compounds from S. zenkeri bark to reduce chronic hyperglycemia while preventing its associated complications in a streptozotocin-induced diabetic Wistar rat model.

2. Materials and methods

2.1. Plant material

The barks of S. zenkeri were collected in the city of Douala (Littoral Region Cameroon) in October 2020. Subsequently identified at the National Herbarium of Cameroon in comparison with Bullock material N° 687 from the Herbarium collection N°39,986 HNC. The barks were transported to the Laboratory of Nutrition and Nutritional Biochemistry (LNNB) of the Department of Biochemistry of the University of Yaoundé I, where they were washed, cut into small pieces, dried in the open air until obtaining constant weight and ground in a Moulinex (Philips Stay Fresh HR3752/00) until a powder is obtained.

2.2. Preparation of the aqueous extract of S. Zenkeri bark(AESZ)

Five hundred grams (500g) of powder were dissolved in 5 L of distilled water (1:10 weight/volume) and macerated at room temperature for 24h The supernatants were collected and then filtered using Whatman No. 1 paper (Whatman International Limited, Ken, England) and the filtrates obtained were dried in an oven (WGLL-65BE) at 60 °C. for 72 h to evaporate the solvent which made it possible to obtain the AESZ.

2.3. Quantification of bioactive compounds of interest

The bioactive compounds that were determined in the extract were: total polyphenols, flavonoids and alkaloids.

2.3.1. Assay of total polyphenols

The extract polyphenol content was evaluated using the protocol described by Singleton and Rossi [20]. Thirty microliters (30 μL) of extract (1 mg/mL) prepared in 95% ethanol was added to 1 mL of Folin's solution (0.2 N), the absorbance was measured at 750 nm after 30 min incubation at 25 °C using a spectrophotometer. Gallic acid was used as the standard. The content of total polyphenols was expressed in micrograms of gallic acid equivalents per milligram of extract (μg of EAg/mg of extract).

2.3.2. Assay of flavonoids

The extract flavonoid content was evaluated using the protocol described by Aiyegoro and Okoh (2010). A volume of 1 mL of extract prepared in 95% ethanol at a concentration of 1 mg/mL was added to 1 mL of AlCl3 (10%), 1 mL of potassium acetate (1 M) and 5.6 mL of distilled water. After 30 min of incubation at 25 °C, the absorbance was read at 430 nm using a spectrophotometer. Quercetin was used as the standard. Flavonoid content was expressed as micrograms of quercetin equivalents per milligram of extract (μg Quer/mg extract).

2.3.3. Assay of alkaloids

The quantification of alkaloids in the extract was carried out using the protocol described by Singh et al. (2004). A mass of 100 mg of extract powder was subjected to extraction in 10 mL of 95% ethanol, then filtered and centrifuged at 5000 g for 10 min. In the supernatant obtained, 1 mL was taken to which was added 1 mL of [FeCl3 (0.025 M) + HCl (0.5 M)] and 1 mL of 1.10 Phenanthroline (0.05 M) prepared in ethanol. The mixture obtained was incubated in a water bath for 30 min with the temperature maintained at 70 ± 2 °C. The absorbance of the red color of the complex formed was read at 510 nm against white. Quinine was used as the standard. The alkaloid content was expressed in micrograms of quinine equivalents per milligram of extract (μg of Qui/mg of extract).

2.4. Experimentation

2.4.1. Animals

The animals consisted of twenty (20) male Wistar rats, with an average weight of 240 ± 10 g, purchased from the Laboratory of Biology and Animal Physiology of the University of Yaoundé I. None of these animals had been subject to previous experiences and showed no signs of abnormalities. The rats were acclimatized for 7 days in the animal facility of the Laboratory of Nutrition and Nutritional Biochemistry of the University of Yaoundé 1 at the ambient temperature of the environment and subjected to a day-night cycle of 12 h in good ventilation conditions and natural lighting. They were placed in plastic basins covered with mesh and received daily a normal diet and tap water at will. The litter was made of sawdust renewed every two days to ensure good hygienic conditions. The experimental protocol and the maintenance of the laboratory animals were carried out in accordance with the standard ethical guidelines for the use of laboratory animals and care as described in the guidelines of the European community and the ethics committee of the Faculty of Sciences of the University of Yaoundé I.

2.4.2. Design of the experimental protocol

After 7 days of acclimatization, the rats were divided according to the average weight into 2 groups: a NC group (negative control) consisting of 5 rats and a batch of 15 rats subject to induction of

hyperglycemia. The induction was done according to the method of Al-Shamaony et al. [21] with some modifications. Hyperglycaemia was induced by intraperitoneal injection of a dose of 45 mg/kg of body weight of streptozotocin dissolved in citrate buffer (100 mM; Ph 4.5; 150 mM NaCl). 1 h after administration of the STZ, the animals received glucose water (10%) in order to avoid hypoglycemic shock following the administration of the STZ and 2 h after food. Screening was performed 48 h after injection of STZ in fasting animals by measuring blood glucose by the reactive strip method using a "One Touch Ultra" glucometer. Rats with a blood sugar level greater than or equal to 200 mg/dL were considered ashyperglycaemic (15 rats) and used for the rest of the experiment [22]. Subsequently, the rats were divided according to the average blood sugar into 4 groups of 5 rats as follows:

- Group I (negative control rats): non-hyperglycemic rats receiving distilled water by daily gavage;
- Group II (positive control): untreated hyperglycaemic rats receiving distilled water by daily gavage;
- Group III (trial): hyperglycemic rats receiving AESZ by daily gavage at a dose of 300mg/kgBW;
- Group IV (reference): hyperglycaemic rats receiving metformin by daily gavage at a dose of 20mg/kgBW.

The volume of administration was 5 mL/kgBW. The treatment lasted 21 days and was performed every morning by esophageal gavage using a probe. During the experiment, the weight of the rats was taken twice a week while the blood glucose levels were taken for each group at regular time intervals of seven (7) days until twenty-first (21st) days. The calculation of the percentage changes in blood glucose and weight was performed as follows:

$$\text{Glycemic}\ (\%) = \frac{\boldsymbol{Glycemic}(\boldsymbol{GX}) - \boldsymbol{Glycemic}\ (\boldsymbol{G}0)}{\boldsymbol{Glycemic}\ (\boldsymbol{G}0)} \times 100$$

G0: Blood glucose at day = 0, Gx: Blood glucose after each 7 days

$$\text{Weight variation}\ (\%) = \frac{(\boldsymbol{Finalweight} - \boldsymbol{Initialweight})}{\boldsymbol{Finalweight}\ (\boldsymbol{g})} \times 100$$

2.4.3. Euthanasia of animals and preparation of biological samples

At the end of the treatment, the animals were fasted for 12 h, after light anesthesia with chloroform, the rats were euthanized by cardiac puncture. The blood was collected in the dry tubes for the preparation of the serum and in the EDTA tubes for the numbering of the blood formula, the preparation of the plasma and the hemolysate. For plasma preparation, the blood was allowed to stand for 4 h at room temperature (25 °C) and then centrifuged at 900 g for 10 min. Plasma was collected, aliquoted and stored in Eppendorf tubes at −20 °C for biochemical assays. Subsequently, a volume of 100 μL of pellet was introduced into conical tubes then washed in 2 mL of an NaCl solution (0.9%) and centrifuged at 900 g for 10 min, at room temperature. This process was repeated twice. Hemolysis of erythrocytes is carried out by adding 2 mL of distilled water followed by centrifugation at 900*g* for 10 min at 25 °C. The supernatant (hemolysate) was collected, aliquoted and stored at −20 °C. The liver removed by dissection was washed in the NaCl solution (0.9%), drained, weighed and ground separately in a mortar; then the ground material was homogenized in 10% (weight/volume) of phosphate buffer (0.1 M; pH 7.4). The homogenates obtained were stored at −20 °C.

2.4.4. Effect on oxidative stress

The markers evaluated were erythrocyte catalase according to the method described by Sinha [23] and hepatic malondialdehyde (MDA), a marker of lipid peroxidation according to the method described by Ref. [24].

2.4.5. Effect on immunological parameters

Hematological parameters were analyzed using a Beckman Coulter Medonic. The parameters analyzed were: red blood cell, White Blood Cells, Neutrophils, Monocytes, Lymphocytes and platelets.The C-reactive protein (CRP) assay was performed according to the protocol of Ritchie et al. [25], based here on turbidimetric measurements.

2.4.6. Effect on plasma lipid profile

Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-c) were evaluated in plasma using the chronolab kits. Low density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) were determined from the formula of Friedewald, Levy and Friedrickson [26].

$$\text{LDL} - \text{c}\ (\text{mg/dL}) = \text{TC} - (\text{HDL} - \text{c} + \text{TG}/\ 5)$$

$$\text{VLDL} - \text{c}\ (\text{mg/dL}) = \text{TG}/\ 5$$

2.4.7. Effect on atherogenic risks

The lipid profile parameters were used to evaluate the Atherogenic Index of Plasma (AIP), Atherogenic Coefficient (AC), Cardiac Risk Ratio (CRR) and Cardioprotective Index (CPI) according to the following formulas: [27–29], (Oršoli'et*al.*, 2014).

$$\text{CRR1} = \frac{\text{TG}}{\text{HDL} - \text{C}},\ \text{CRR2} = \frac{\text{LDL} - \text{c}}{\text{HDL} - \text{C}}$$

$$\text{AC} = \frac{\text{TG} - \text{HDL} - \text{C}}{\text{HDL} - \text{C}}$$

$$\text{AIP} = \log\frac{\text{TG}}{\text{HDL} - \text{C}}$$

$$\text{CPI} = \frac{\text{HDL} - \text{C}}{\text{LDL} - \text{C}}$$

2.5. Data processing and analysis

Results were expressed as the mean ± standard error of the mean and percent change. Statistical analysis was performed using SPSS (Statistical Package for Social Science) version 20.0 for Windows. The one-way ANOVA (Analysis Of Variance) test followed by a Tukey post-hoc test was used for the comparison between the groups. Results with a $p < 0.05$ were considered significantly different. The Excel software was used to process the results and draw the graphs.

3. Results

3.1. Content of bioactive compounds of interest ofAESZ

The polyphenol, flavonoid and alkaloid contents of AESZ are shown in Table 1 below. It appears that the extract showed significantly high levels of polyphenols (177.27 μg of EAg/mg of extract) and alkaloids (217.41 μg of EQui/mg of extract).

3.2. Influence of AESZ on the evolution of body weight

Fig. 1 shows the result of the effect of the extract on the change in

Table 1
Contents of polyphenols, flavonoids and alkaloids in the extract.

Bioactive Composés	Polyphenols (μg of EAg/mg)	Flavonoids (μg of EQuer/mg)	Alkaloids (μg of EQui/mg)
AESZ	177,27 ± 2,31	24,44 ± 0,14	217,41 ± 0,96

AESZ: Aqueous extract of the barks of S. zinkeri; EQer: Equivalent Quercetin; EAg: Gallic acid equivalent; EQui: Quinine equivalent.

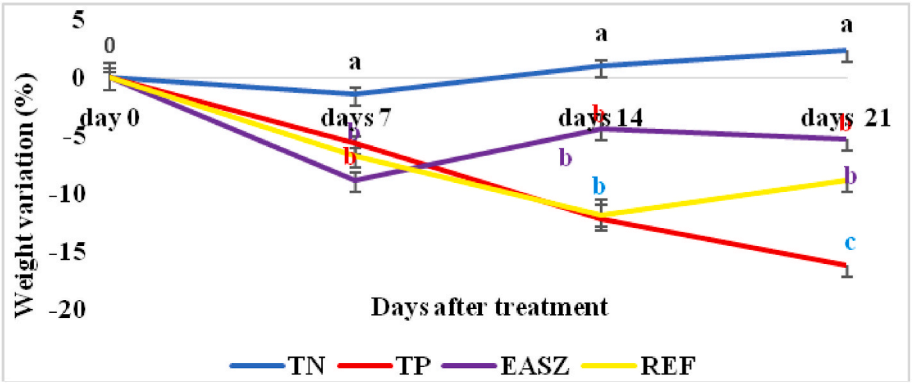

Fig. 1. Effect of AESZ on the weight variation of experimental rats.
Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AESZ: rats treated with 300 mg/kg of S. zenkeri bark; REF: Reference (diabetic rats + 20 mg/kg of metformin. The points assigned different letters (a, b, c, d) are significantly different ($P < 0.05$).

body weight expressed as a percentage change. It was noted throughout the experiment, a significant and progressive loss ($p < 0.05$) of body weight in the PC group compared to the NC group. However, the administration of the extract resulted from the 2 nd week, a weight gain ($p < 0.05$) compared to the PC group. This weight gain was also noted with the reference group from the 3rd week.

3.3. Influence of AESZ on hyperglycaemia after 21 days of treatment

Fig. 2 shows the result of the change in glycaemia during the treatment, expressed as mean glycaemia and as a percentage change. The injection of streptozotocin caused a significant increase in blood sugar in the PC group compared to the NC group (478.93 mg/dL versus 70 mg/dL). After 21 days of treatment, the extract caused a significant decrease in blood sugar from the 14th day (−30.22%) to the 21st day (−26.59%). The reference (metformin) was more effective than extract which resulted in a reduction of −30.63% on the 21st day.

3.4. Effect of the extract on endogenous oxidative status

Fig. 3 shows the results of the effect of the extract on catalase activity and lipids peroxidation at the level of the liver. There is a significant decrease ($P < 0.05$) in erythrocyte catalase activity (Fig. 3a), as well as an increase in hepatic MDA levels (Fig. 3b) in rats of the PC group compared to the NC group. However, administration of the extract resulted in a significant increase in catalase activity and a significant reduction of hepatic MDA levels. Compared to the reference group, the extract showed significantly lower effect on lipid peroxidation.

3.5. Effect of AESZ on some inflammatory parameters

3.5.1. Effects of the extract on C-reactive protein concentration

Fig. 4 shows the effect of AESZ on C-reactive protein concentration. There is a significant increase ($P < 0.05$) in the serum concentration of C-reactive protein in the PC group compared to the NC group (17.06 versus 8.99 µg/dL). Administration of the extract led to a significant drop in this concentration compared to the PC group (10.12 versus 17.06 µg/dL). The effect of the extract was comparable to that obtained

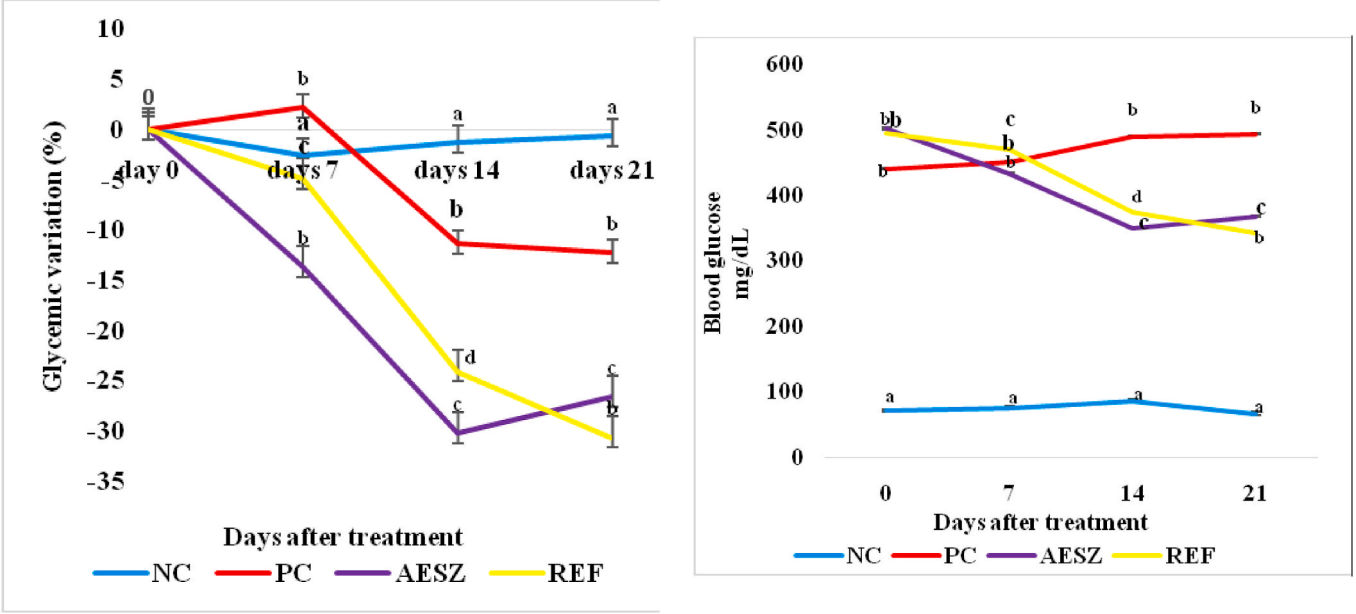

Fig. 2. Effect of EASZ on chronic hyperglycemia.
The values are expressed as the mean ± standard deviation, the values in parentheses represent the variations in blood glucose levels as a percentage; Values assigned different letters (a, b, c, d) are significantly different ($P < 0.05$).

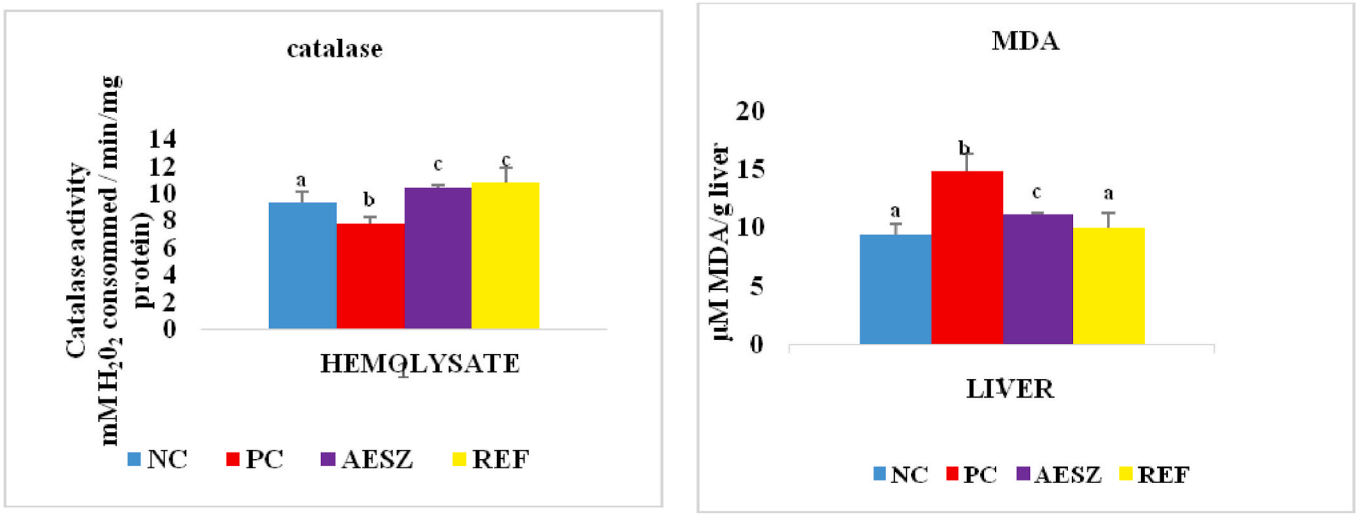

Fig. 3. Effect of AESZ on endogenous oxidative status.
Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AESZ: rats treated with 300 mg/kg of S. zenkeri bark; REF:rats treated with 20 mg/kg of metformin. The points assigned different letters (a, b, c, d) are significantly different ($P < 0.05$).

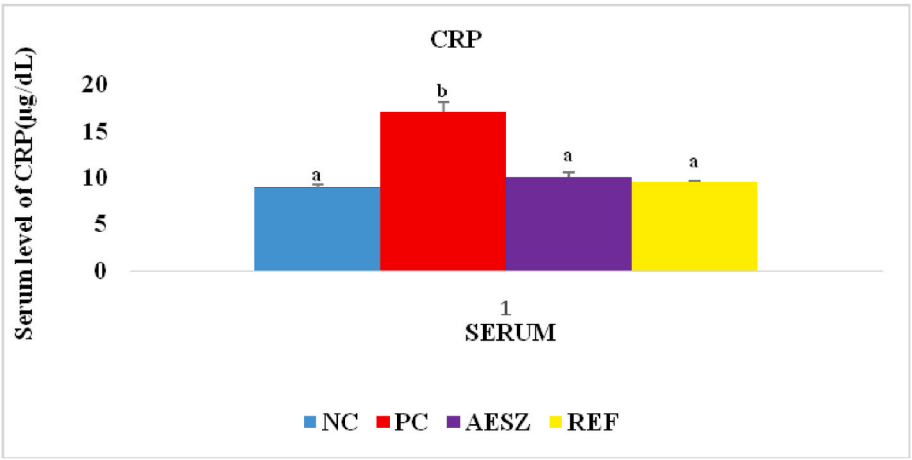

Fig. 4. Effects of the extract on serum C-reactive protein concentration.
Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AESZ: rats treated with 300 mg/kg of S. zenkeri bark; REF:rats treated with 20 mg/kg of metformin. The points assigned different letters (a, b, c, d) are significantly different ($P < 0.05$).

with the reference drug (metformin).

3.5.2. *Effects of the extract on hematological parameters*

Fig. 5 shows the result of the effect of AESZ on hematological parameters. It shows that, compared to the NC group, the PC group showed a significant decrease of red blood cell and increase in white blood cells (10.88 against 16.04 x103/µL), lymphocytes (72.87 against 82.53%), monocytes (2.76 against 15.87%) and Neutrophils (14.8 against 22.1%). However, the administration of AESZ resulted in a significant decrease in these hematology parameters compared to the PC group.

3.6. *Effect of the extract on the plasma lipid profile*

It appears from Table 2 that hyperglycemia led to an alteration of lipid metabolism in animals, characterized by a significant increase ($P < 0.05$) in the content of triglyceride, total cholesterol, VLDL-cholesterol and LDL-cholesterol as well a decrease in HDL-cholesterol in the untreated group (positive control) compared to the normal group (negative control). However, the administration of the extract led to a positive improvement in these parameters, in particular the reduction in triglycerides, VLDL-cholesterol and LDL-cholesterol as well as an increase in HDL-cholesterol, just like the reference molecule.

3.7. *Effect of the extract on atherogenic risks*

Table 3 represents the effect of the extract on Atherogenic risks. It appears that the RCC, AC and AIP were higher in the rats of the positive control group compared to the normal group while CPI was lower in the untreated group compared to the normal group ($p < 0.05$). The administration of the extract significantly reduced ($p < 0.05$) the CRR, AC and AIP while increasing CPI. The effect of the extract was comparable to that of the reference medicine.

4. Discussion

In a bid to tip the rising crescent of diabetes and associated complications, the search for new, more effective bioactive compounds is increasingly topical and indispensable (Rashmi et al., 2017).This is why the search for compounds with multiple mechanisms of action are increasingly emerging. The present work aimed to evaluate the effect of

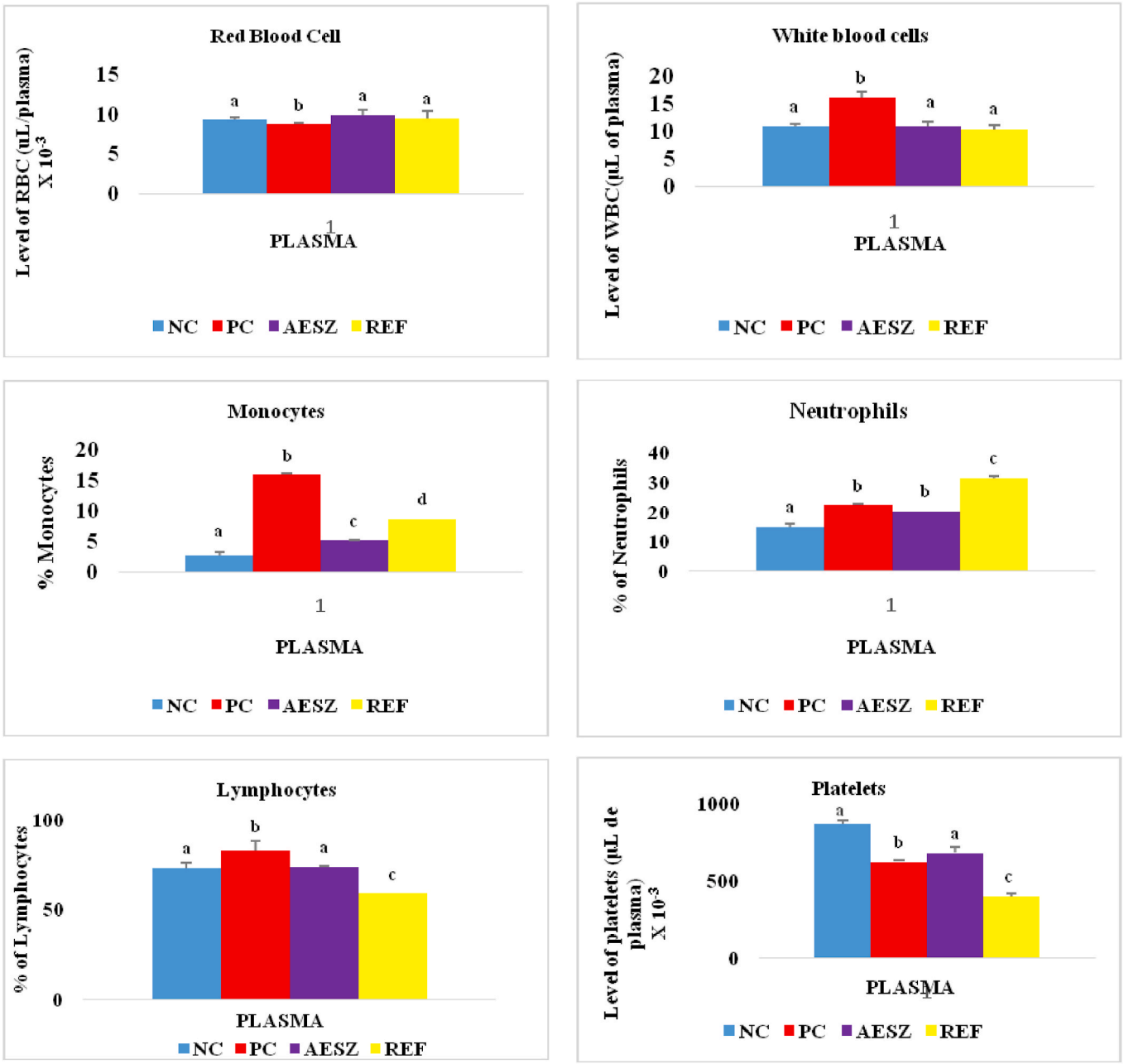

Fig. 5. Effect of extract on hematological parameters.
Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AESZ: rats treated with 300 mg/kg of S. zenkeri bark; REF:rats treated with 20 mg/kg of metformin; WBC: White blood cells; RBC: Red blood cells. The points assigned different letters (a, b, c, d) are significantly different ($P < 0.05$).

the aqueous extract of S. zenkeri bark on chronic hyperglycemia and some metabolic disorders associated with it such as oxidative stress, inflammation and dyslipidemia in a Wistar rat model of streptozotocin-induced hyperglycemia. To achieve our objectives, we have quantified certain bioactive compounds that can interact with the mechanisms regulating hyperglycemia and the associated metabolic disorders. Indeed, the ability of polyphenols, flavonoids and alkaloids has been demonstrated to reduce hyperglycemia whether postprandial or fasting; as well as preventing the disorders associated with it such as oxidative stress, inflammation and dyslipidemia (Liza and Johannes, 2012; [30]. We noted considerable contents of total polyphenols, flavonoids and alkaloids in the aqueous extract of the barks of S. zenkeri (Table 1); as demonstrated by Abdou [31] with the methanolic extract of the bark of this same plant.

During the experiment, the administration of streptozotocin caused a progressive loss of weight on the untreated hyperglycemia group compared to the normoglycemic control group (Fig. 1); which confirms the success of the induction model. Indeed, Zhang et al. [32] showed that the induction of chronic hyperglycemia in rats by streptozotocin was accompanied by a progressive loss of body weight. This weight loss can be explained by the result of a catabolism of structural lipids and proteins due to the lack of carbohydrates that have been used as an energy source. After 21 days of experimentation, the extract significantly limited weight loss compared to the untreated hyperglycemic group. These beneficial effects of the extract could be due to its high in polyphenols. Indeed, although it is established that phenolic compounds have the ability to reduce overweight by inhibiting the expression of genes involved in lipogenesis, digestion and absorption of lipids [33]. The limitation of weight loss observed in the group treated with our extract can be explained by the fact that; phenolic compounds in

Table 2
Plasma lipid profile.

	NC (negative control)	PC (positive control)	AESZ (treated group)	Reference group
TG (mg/dL)	74,61 ± 0,9[a]	122,71 ± 1,0[b]	59,69 ± 0,06[c]	61,98 ± 2,52[a]
TC (mg/dL)	119,26 ± 1,82[a]	170,58 ± 3,72[a]	86,31 ± 2,2[b]	177,04 ± 8,64[a]
VLDL-C (mg/dL)	14,92 ± 0,44[a]	24,54 ± 0,80[b]	10,33 ± 1,04[c]	11,59 ± 4,54[a]
LDL-C (mg/dL)	94,27 ± 1,18[a]	163,35 ± 0,38[b]	9,71 ± 0,64[c]	31,76 ± 1,82[d]
HDL-C (mg/dL)	40,90 ± 1,29[a]	31,76 ± 3,72[b]	57,07 ± 1,05[c]	41,66 ± 1,74[a]

Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AESZ: rats treated with 300 mg/kg of S. zenkeri bark; Reference: diabetic rats +20 mg/kg of metformin. TG: Triglycerides, TC: Total cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol. The values assigned different letters (a, b, c, d) are significantly different ($P < 0.05$).

Table 3
Atherogenic risks.

	NC (negative control)	PC (positive control)	AESZ (treated group)	Reference group
CRR1	1,86 ± 0,16[a]	3,86 ± 1,04[b]	1,04 ± 0,85[c]	1,39 ± 1,01[a]
CRR2	2,36 ± 0,05[a]	5,14 ± 0,85[b]	0,16 ± 0,06[c]	0,76 ± 0,55[c]
AC	73,61 ± 0,45[a]	121,71 ± 2,66[b]	0,04 ± 0,77[c]	56,98 ± 1,24[a]
AIP	0,27 ± 0,001[a]	0 ,58 ± 0,05[b]	0,01 ± 0,001[c]	0,14 ± 0,001[d]
CPI	0,42 ± 0,02[a]	0,19 ± 0,01[b]	6,12 ± 0,03[c]	1,31 ± 0,07[c]

Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AESZ: rats treated with 300 mg/kg of S. zenkeri bark; Reference: diabetic rats +20 mg/kg of metformin. CRR: Cardiac Risk Ratio, AC: Atherogenic Coefficient, AIP: Atherogenic Plasma Index, CPI: Cardio-Protective Index. Values assigned different letters (a, b, c, d) are significantly different ($P < 0.05$).

addition to their role in activating lipolysis; also play a role in regulation of lipid metabolism. Like the extract, the reference drug (metformin) also limited this weight loss from the 14th day, which suggests that this antidiabetic drug could also play a role in regulation of lipid metabolism. The evaluation of the effect of the extract on chronic hyperglycemia and its complications is carried out in an animal model of hyperglycemia induced by streptozotocin. Indeed, streptozotocin is the most widely used chemical in the induction of diabetes [34]. It's a nitrosated glucosamine that causes a selective cytotoxic effect on β-cells of islets of Langerhans [34]. Thus, its administration caused a sudden increase in glycaemia in all groups of animals (PC, AESZ and REF) compared to the normoglycemic control group. The administration of the extract significantly reduced blood sugar from the 7th day of treatment. These results could be explained by the presence of alkaloids in the extract which have the ability to stimulate insulin secretion by residual beta cells and to inhibit glucose-6-phospatase and phosphoenolpyruvate carboxykinase; keys enzymes of gluconeogenesis [15,16]. Moreover, postprandial hyperglycemia has being strongly involved in the occurrence of chronic hyperglycemia; the ability of the extract to lower blood sugar could also partly due to the ability of the alkaloids found in the extract to inhibit carbohydrate digestion enzymes (β-galactosidase, α-glucosidase, α-amylase and invertase) (Znifeche., 2019). Dailleur, Etoundi et al. [18] reported the anti-amylase potential of aqueous extracts from the fruits of S. zenkeri.

Hyperglycemia is associated with many microvascular complications, which are favored and strongly proportional to oxidative stress and inflammation [35,36]. Oxidative stress is a factor involved in the development of vascular lesions leading to the complications associated with hyperglycemia. It is involved in abnormal activation of pro-inflammatory signaling pathways leading to increased secretion of pro-inflammatory cytokines such as IL-6, TNF-α; materialize by production of hepatic CRP [37]. On the other hand, inflammation accelerates the production of ROS and decreases the antioxidant defense capacity, accentuating oxidative stress. This is why the study of oxidative stress parameters in erythrocytes and liver shows a very remarkable decrease erythrocyte catalase and a significant increase in hepatic lipid peroxidation in the untreated hyperglycemic group compared to the normoglycemic control group. Administration of the extract improved andogenic antioxidant status by increasing catalase activity and reducing MDA levels (Fig. 4). These effects could be attributed to the presence of flavonoids in the extract which, by the redox potential of their OH groups, would be able to yield a proton and/or an electron, thus trapping free radicals able to cause molecular and cellular oxidative damage [38]. On the other hand, these effects would probably be attributed to the presence of cofactors (iron and manganese) in our extract involved in catalase activity. Moreover, the work of [31] has shown that the fruits and barks of S. zenkeri are rich in microelements including iron and manganese. The ability of the extract to reduce oxidative stress would make it possible to limit upstream the activation of pro-inflammatory signaling pathways and, in turn, limit the inflammatory disorders associated with a state of chronic hyperglycemia. The emerging role of inflammation in the pathophysiology of diabetes and associated complications has prompted growing interest in targeting inflammation to improve disease prevention and control [11]. Administration of the extract resulted in a reduction of inflammation; materialize by a significant decrease in CRP (Fig. 4). This effect could be attributed to the presence of polyphenols in our extract, mainly flavonoids with an anti-inflammatory attribute by inhibition of regulatory enzymes such as kinases (PKC, PI3kinase and tyrosine kinases) involved in the inflammatory response [39]. In addition, the studies conducted by Sahar and Mahdieh [40] showed that the polyphenols present in grapes have the ability to inhibit proinflammatory cytokines, thus decreasing the secretion of CRP. An inflammatory state is associated with a high production of inflammatory cells [39]; this is why the determination of inflammatory cells (white blood cells, lymphocytes, monocytes), and red blood cells in this study; revealed a significant decrease in red blood cells and an increase in those of white blood cells, including lymphocytes and monocytes; in the untreated hyperglycemia group compared to the normoglycemic control group. The fall in red blood cells and the increase in those of white blood cells in untreated hyperglycemic rats could be the result of anemia or the onset of the glycosylation process such as observed in diabetics with chronic hyperglycemia [41]. Indeed, a variation in hematological parameters in a subject is synonymous with an anomaly in the functioning, morphology or metabolism of erythrocytes, leukocytes and platelets [42]. The non-variation of the hematological parameters in the treated rats compared to the normoglycemic control rats could mean that our extract would not affect the hematological parameters of the treated hyperglycemic rats, but would rather act as a regulator of these parameters.

Hyperglycaemia is also associated with many macrovascular complications through dyslipidemia which are consecutive to insulin functioning disorders [43]. This justified the choice of evaluating the effects of AESZ on lipid profile markers. The extract lowered triglyceride, total cholesterol, LDL-c and VLDL-c levels while increasing HDL-c levels (Table 2). These hypotriglyceridemic and hypocholesterolemic properties of the extract could be still attributed to their content in polyphenols, which are capable of stimulating lipoprotein lipase and inhibiting HMG-CoA reductase which are key enzymes respectively involved in the metabolism of triglycerides and the synthesis of cholesterol [44]. Dyslipidemia associated with diabetes increases the risk of cardiovascular disease, a risk that can be assessed by several indices including: CRR, AIP, AC and CPI [45]; NCEP, 2002). The extract reduces the cardiovascular risk materialized by the reduction of CRR, AC and AIP and an increase in CPI (Table 3). Indeed, these different indices predict cardiovascular risk better than LDL-c or HDL-c alone. This effect would be the consequence of the beneficial effects of the extract on the

markers of the lipid profile [46–50].

5. Conclusion

The results of this study show that the aqueous extract of Scorodophloeuszenkeri bark reduces chronic hyperglycemia and prevents the development of its macro and microvascular complications upstream. This work highlights the beneficial effects of Scorodophloeuszenkeri traditionally used in the management of diabetes. In order to refine our work, we propose to evaluate the mechanisms of action of the extract that may be associated with its hypoglycemic activity, to evaluate other inflammatory markers associated with a diabetic state such as prostaglandins, pro-inflammatory cytokines (TNF-α, IL-1 and IL-6) and to characterize the bioactive compounds present in our extract responsible for the antidiabetic effects.

CRediT authorship contribution statement

Martin Fonkoua: Formal analysis, Data curation, Writing – original draft. **Marielle Zali Ze:** Formal analysis, Data curation. **William Arnold Tazon:** Formal analysis, Data curation, Writing – original draft. **Janvier Youovop:** Formal analysis, Data curation. **Guy Takuissu Nguemto:** Formal analysis, Data curation.

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