

ASSESSMENT OF THE ANTIOXIDANT AND ANTI-DIABETIC ACTIVITY OF *ANNONA SENEGALENSIS* AND *HALLEA LEDERMANNII* IN ALLOXAN-INDUCED DIABETIC RATS

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Abstract

In the present study the antioxidant and antidiabetic effects of the aqueous extracts of dry leaves of *Annona senegalensis* (Annonaceae) and *Hallea ledermannii* (Rubiaceae) were carried. For antioxidant activities the data obtained show that the both extracts contained flavonoids and polyphenols which would be at the origin of their antioxidant properties. The antidiabetic activities are carried out in 13 weeks in alloxan-induced diabetic Wistar rats which received daily the doses of glibenclamide (10^{-2} g/kg) and those of *Annona senegalensis* (100 and 200 mg/kg) and *Hallea ledermannii* (200 and 400 mg/kg). The findings showed the goods effects of these plants on diabetic rats hyperglycemia. These treatments do not disturb the lipid profile after 91 days of daily administration. The antidiabetic properties in alloxan-induced diabetic rats would be due to the antioxidant activities through the bioactive compounds as flavonoids, saponosides, triterpenes and tannins present in both extracts. Therefore, these plants are good candidates for further studies.

Keywords: Antidiabetic properties, *Annona senegalensis*, *Hallea ledermannii*, Antioxidant activity, Alloxan, Glibenclamide

Introduction

Diabetes mellitus is a set of syndromes associated with a disruption of carbohydrate metabolism (1). It occurs when the body is unable to produce enough insulin (type 1) or use insulin effectively (type 2) (2, 3, 4) and are characterized by either chronic hyperglycemia or fasting blood glucose greater than 1.26g/l (5). It is one of the most prevalent noncommunicable diseases in the world with approximately 422 million patients in 2014 (6). According to Shaw et al. (7), this number would be estimated at 7.7% of the world's population by 2030. Shaw et al. (7) also estimates that more than 75% of this increase will occur in developing countries as a result of changes in the way of life of the population and urbanization. Diabetes is responsible for more than 80% of deaths of diabetic patients in low- and middle-income countries (8). According to estimates by Mbanya et al. (9), the prevalence of diabetes in sub-Saharan Africa is expected to reach 4.7% in 2030. In Côte d'Ivoire, the prevalence of diabetes mellitus was estimated in 2014 at 9.6%, according to the International Diabetes Federation (FID) (10). For economic reasons, patients in developing countries combine ethno-medical and pharmaceutical treatments to hope for cure (11, 12). It is also known that more than 80% of the African population uses medicinal plants (13), which are invaluable resources for the pharmaceutical industry (14), to overcome health care system. It is in this perspective that we have undertaken to study the effects of the aqueous extracts of *Annona senegalensis* (Annonaceae) and *Hallea ledermannii* (Rubiaceae), two plants of the African traditional pharmacopoeia, used in the treatment of diabetes (15, 16, 17). *Annona senegalensis*, a medicinal plant used to treat a large number of pathologies of infectious origin (18), also has antiparasitic activity on a resistant strain of *Plasmodium falciparum* (19). In addition, antibacterial activities have been reported by More et al. (20). *Hallea ledermannii* is used as a local anesthetic to lower blood pressure and cause disorders in the lymphatic system of the intestine (21). This plant also has antimicrobial and antioxidant activity (22).

The aim of this work is to evaluate the antidiabetic effects of *Annona senegalensis* and

Hallea ledermannii extracts in diabetic rats in order to promote traditional pharmacopoeia

Material

Plant material

The plant material consists of dry leaves of *Annona senegalensis* (Annonaceae) and *Hallea ledermannii* (Rubiaceae) harvested, respectively, in Bouaflé and yopougon (neighborhood in Abidjan), Côte d'Ivoire. They were identified at the National Floristic Center of the Félix Houphouët Boigny University (Côte d'Ivoire) by the late Professor Aké Assi. Samples of *Annona senegalensis* (Annonaceae) and *Hallea ledermannii* (Rubiaceae) are kept, respectively, under the herbaria numbers 9,809 Lamto 06/12/1967 and 2,538 Forest of the banco 14/10/1954.

Extraction process

To three hundred (300) grams of dried leaves of *Annona senegalensis* or *Hallea ledermannii*, which were cut into pieces, 1.5 l of distilled water is added and the whole is brought to a boil for 1 hour. The decoctate obtained is filtered several times on hydrophilic cotton. The decoction is dried in an oven at 60°C. After drying, we obtained the powders of *Annona senegalensis* and *Hallea ledermannii* that we stored in the fridge.

Animal material

The experiments were carried out on rats of the species *Rattus norvegicus* of Wistar strain weighing between 200 and 250 g and bred in the animal house of Biosciences of the Felix Houphouët-Boigny University, at the ambient temperature (25°C). These rats had access to food and water condition before the start of experiment. All procedures were approved by the ethical committee University Felix Houphouët-Boigny, and conducted in accordance with the national government principles accepted for the use of laboratory animal and care.

Methods

Spectrophotometric determination of total polyphenols contents in aqueous extracts of *Annona senegalensis* and *Hallea ledermannii*

The method of Wood et al. (23) was used for the determination of total polyphenols. A volume of 2.5 ml of Folin-Ciocalteu reagent diluted to the tenth was added to 30 µl of extract. The mixture was kept for 2 minutes in the dark at room temperature, then 2 ml of sodium carbonate solution (75 g/l) was

added. Then, the mixture was placed for 15 minutes in a water bath at 50 °C and then rapidly cooled. Absorbance was measured at 760 nm, with distilled water as white. A calibration line was carried out with gallic acid at different concentrations. The analyzes were carried out in triplicate and the concentration of polyphenols was expressed in grams per liter of gallic acid equivalent extract (g l^{-1} , Eq AG).

Spectrophotometric determination of total flavonoids in aqueous extracts of *Annona senegalensis* and *Hallea ledermannii*

The total flavonoid assay was performed according to the method described by Marinova et al. (24). In a 25 ml flask, 0.75 ml of 5% (w/v) sodium nitrite (NaNO_2) was added to 2.5 ml of extract. To the mixture, 0.75 ml of 10% (w/v) aluminum chloride (AlCl_3) was added, and then the whole was incubated for 6 minutes in the dark. Once this time had elapsed, 5 ml of sodium hydroxide (1N NaOH) was added thereto and the volume was made up to 25 ml. The preparation was vigorously shaken before the determination of total flavonoids at 510 nm with the UV-visible spectrophotometer. The tests were carried out in triplicate and the total flavonoid content was expressed in grams per liter of quercetin equivalent extract (g l^{-1} EqQuer.) A calibration line was made with quercetin at different concentrations.

Determination of antiradical activity by the DPPH (2, 2'-diphenyl-1-picrylhydrazyl) test of aqueous extracts of *Annona senegalensis* and *Hallea ledermannii*

The measurement of the antiradical activity of the plant extracts was carried out by the test with 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of Parejo et al. (25). A range of concentrations (0-200 $\mu\text{g/ml}$) of plant extract is prepared in methanol. A volume of 2.5 ml of this solution is mixed with 2.5 ml of DPPH (100 μM) also prepared in methanol. After homogenization, the mixture is incubated at room temperature (25 °C.) in the absence of light. After 15 minutes of incubation, the absorbance is read at 517 nm against a "white" which contains only methanol. The percentage of inhibition of the DPPH radical is calculated according to the following equation: Inhibition of the DPPH (%) = $(1 - (\text{OD test}/\text{OD white})) \times 100$. The IC₅₀ which is the concentration of plant extract responsible for

50% inhibition of the DPPH radicals is determined on the graph representing the percentage inhibition of the DPPH as a function of the concentrations of the extracts.

Study of the effects of an aqueous extract of *Annona senegalensis* and *Hallea ledermannii* in alloxan-induced diabetic rats

In this study, the start of treatment with the aqueous extract or reference product glibenclamide or with distilled water started 24 hours after the confirmation of diabetes. Seventy (70) diabetic rats, divided into two groups of 35 animals are used respectively for the 28 and 91 day tests. For each type of experiment, 35 rats are divided into 7 lots of 5 rats in the cages. After determining their blood glucose level in their 18 h fasting without water, these animals, orally received a reference solution glibenclamide (10^{-2} g/kg bw), the aqueous extract of *Annona senegalensis* (100 mg/kg and 200 mg/kg bw) and the aqueous extract of *Hallea ledermannii* (200 mg/kg and 400 mg/kg bw). The substances tested are administered daily over a period of 4 and 13 weeks.

Measurements of blood glucose, weight and blood samples are taken on day 0 (1st day of treatment) and after 2 (day 14); 4 (J28); 8 (J56) and 13 (J91) weeks.

Thus, for the evaluation of the effects of substances, diabetic animals are distributed as follows:

- Lot 1, nondiabetic control rat: these rats received daily by gavage 2 ml of distilled water;
- Lot 2, untreated diabetic: these rats were received 2ml of distilled water;
- Lot 3, Diabetic + Gli10: these rats which received daily by gavage 2 ml of a glibenclamide solution dosed at 10^{-2} g/kg bw;
- Lot 4, Diabetic + EAAs100: rats received daily by gavage 2 ml of the aqueous extract of *Annona senegalensis* dosed at 100 mg/kg bw;
- Lot 5, Diabetic + EAAs200: rats received daily 2 ml of the aqueous extract of *Annona senegalensis* dosed at 200 mg/kg bw;
- Lot 6, Diabetic + EAHL200: these rats are treated daily by gavage 2 ml of *Hallea ledermannii* dosed at 200 mg/kg bw;
- Lot 7, Diabetic + EAHL400: these rats were received daily orally, 2 ml of *Hallea ledermannii* assayed at 400 mg/kg bw.

Assaying glycated hemoglobin (hba1c) in the blood of diabetic rats after 91 days of treatment

The glycated hemoglobin assay was performed on the blood of fasted rats for 18 hours after 3 months of daily treatment. These rats were divided into 7 lots (normal control lot, diabetic control lot, glibenclamide-treated lot, EAAs-treated lot 100mg/kg bw, EAAs-treated lot 200mg/kg bw, EAHl-treated lot 200mg/kg bw and lot treated with EAHl 400mg/kg bw), and their blood were collected in purple tubes, through the vein of the tail. The serum is taken from each tube, after centrifugation at 500 rpm and dosed by an automaton (COBAS C 311 Roche diagnoses).

Evaluation of hepatic glucose storage of the extracts

After 91 days of treatment, the animals are sacrificed and a lobe weighing 5 g of liver is taken from each of the rats of each batch, cut into small pieces and ground in 30 ml of 4% trichloroacetic acid. The ground material obtained is put in a test tube and centrifuged at 4500 rpm for 5 min, and the supernatant is recovered. 95% ethanol is then added to the supernatant (ethanol/supernatant, 2v/v), the mixture is stirred and heated in a water bath, slowly to boiling. The glycogen precipitates and the suspension obtained is cooled and centrifuged at 4500 rpm for 10 min. The pellet (glycogen precipitated) is added 2 ml of sulfuric acid (H_2SO_4) at 2.5 N and the tube is heated for 30 min. This step allows the hydrolysis of glycogen to glucose. After the hydrolysis, the tube is cooled and one drop of dinitrophenolphtaleine is added thereto, followed by sodium hydroxide at 2.5 N until a color which turns red-oily. This step neutralizes the acidity of the hydrolyzate. For each sample, the glucose thus formed is assayed, in the presence of the GOD-POD reagent (26), by the colorimetric method of Beer (27). The glucose level is determined using a spectrophotometer (Biolabo, France) at 500 nm.

Clinical Chemistry

Blood was collected on 91st day by after sacrifice of some rats by decapitation after anesthesia with ethyl urethane (28). The blood contained in tubes without anticoagulant, is centrifuged at 3000 rpm for 10 min, in a refrigerated centrifuge (Alresa Orto, Spain) at 4 ° C (29), then the serum is collected and deposited in Eppendorf tubes to be stored in the freezer (0 ° C). Serum cholesterol, triglycerides

(TGs), high density lipoproteins (HDL) and light density proteins (LDL) were measured by biochemical assay kits using auto analyzer Mindray BS 240 (Model 240 BS YX 6A 000234, French).

Macroscopic examination.

The animals of all the groups were sacrificed after the study period and subjected to gross examination. The pancreas will be dissected out for observation and the relative organ weight was calculated as (organ/body weight) x 100 %.

Statistical analysis

The statistical analysis of the values and the graphical representation of the data were carried out using the Graph PadPrism 5 software (San Diego, California, USA). The statistical difference between the results was achieved through the analysis of variances (ANOVA), followed by the Tukey-Kramer multiple comparison test, with a significance threshold $p < 0.05$.

Results

Content of polyphenols and total flavonoids of *Annona senegalensis* and *Hallea ledermannii*

The determination of polyphenols and total flavonoids (Figure 1) was carried out respectively by the spectrophotometric method of Folin-Ciocalteu and that of sodium nitrite ($NaNO_2$).

Figure 1A shows the quantity in mg EqAG/g of polyphenol extract in the aqueous extracts *Annona senegalensis* and *Hallea ledermannii*. These results show that the two aqueous extracts contain polyphenols, with a higher content in the aqueous extract of *Annona senegalensis* (61.73 ± 0.40 EqAG/g) compared to that of *Hallea ledermannii* (32.17 ± 1.13 EqAG/g). These values are statistically different ($p < 0.001$).

The determination of flavonoids in the two extracts (Figure 1B), revealed a higher content of this compound in the preparation of *Annona senegalensis* (3.63 ± 0.03 mg EqQ/g) unlike *Hallea ledermannii* (2.97 ± 0.03 mg EqQ/g). These two values are statistically different ($p < 0.01$).

Anti-radical activity of *Annona senegalensis* and *Hallea ledermannii*

Figure 2 shows the percentage inhibition of the DPPH radical as a function of the concentrations of the reference substance (Quercetin) and aqueous extracts of *Annona senegalensis* (EAAs) and *Hallea ledermannii* (EAHl). This study made it possible to

determine the inhibitory concentrations 50% (IC₅₀) of 7.08, 95.4 and 105 µg/mL respectively for quercetin, EAs and EAH. These values show that quercetin is a potent antioxidant ($p < 0.001$) compared to EAs and EAH extracts. However, among the two aqueous extracts used, *Annona senegalensis* has a better activity but is not statistically different ($p > 0.05$) from that of *Hallea ledermannii*. However, EAs and EAH have dose-dependent effects such as quercetin.

Effects of aqueous extracts of *Annona senegalensis* and *Hallea ledermannii* in diabetic rats

Influence of the effect of *Annona senegalensis* and *Hallea ledermannii* on weight

Figure 12 shows weight changes in diabetic rats at day one (D₀) before treatments, then after two (D₁₄), four (D₂₈), eight (D₅₆) and thirteen (D₉₁) weeks.

At day 0, there are no significant variations ($p > 0.05$) in weight between the different batches of rats (Figure 3A). After two weeks (D₁₄) of treatment, a significant decrease in weight was observed in untreated diabetic controls ($p < 0.01$) and in those treated with *Hallea ledermannii* extract ($p < 0.05$) at the dose of 400 mg/kg bw with respective reductions of 11.27% and 9.71%, compared to the control value (250.3 ± 5.63 g) (Figure 3B). After 4 weeks of treatment (D₂₈), a 17.56% ($p < 0.001$) decrease in body weight was observed in the positive controls compared to the normal control. A decrease in body mass also occurred in rats treated with aqueous extracts of *Annona senegalensis* ($p < 0.05$) and *Hallea ledermannii* ($p < 0.01$) compared to the non-diabetic control rat. The weight of animals treated with glibenclamide increased by 12.98% ($p < 0.05$) compared to the positive control (Figure 3C). At day 56, the weight of glibenclamide treated rats (Gli₁₀) and *Annona senegalensis* extracts (EAs₁₀₀ and EAs₂₀₀ mg/kg bw) was similar to that of the nondiabetic control (Figure 3D). However, weight gains of 16.41% ($p < 0.05$) and 13.41% ($p < 0.05$), were observed respectively in glibenclamide-treated rats and those treated with EAs₁₀₀ compared to the untreated diabetic control. During the same period, decreases of 20.32% ($p < 0.001$), 13.55% ($p < 0.05$) and 14.81% ($p < 0.01$) were observed respectively in rats untreated diabetics and in those treated with EAH₂₀₀ and EAH₄₀₀ compared to non-diabetic control. After 13 weeks (J₉₁) (Figure 3E) of

treatment, the weight of these animals decreased by 20.14% ($p < 0.001$) in the diabetic control and by 11.31% ($p < 0.05$) and 11.17% ($p < 0.05$) in those treated with EAH₂₀₀ and EAH₄₀₀ compared to the negative control. In animals treated with Gli₁₀, EAs₁₀₀ and EAs₂₀₀, respective increases of 18.80% ($p < 0.01$), 15.72% ($p < 0.05$) and 14.48% ($p < 0.05$) were observed compared to untreated diabetic controls.

Effects of *Annona senegalensis* and *Hallea ledermannii* on rat blood glucose

Figure 4 shows the change in blood glucose in diabetic rats during the experimental period. At Day 0 (Figure 4A), the glucose levels of the positive control rats and those treated respectively with glibenclamide and EAs (100 and 200 mg/kg bw) and EAH (200 and 400 mg/kg bw) remained significantly elevated ($p < 0.001$) compared to that of negative control rats. After 2 weeks (D₁₄) of treatment, glycemia remains significantly ($p < 0.001$) elevated in untreated diabetics and in those treated with glibenclamide and aqueous extracts of *Annona senegalensis* (100 and 200 mg/kg bw) and *Hallea ledermannii* (200 and 400 mg/kg bw), compared to non-diabetic control. However, respective decreases in blood glucose were 27.20% ($p < 0.05$) and 25.77% ($p < 0.05$) in the lots treated with glibenclamide and EAs₁₀₀ compared to the diabetic control untreated (Figure 4B). At the day 28, blood glucose levels remained elevated in untreated diabetic animals ($p < 0.001$) and those treated with glibenclamide ($p < 0.05$) and extracts of *Annona senegalensis* ($p < 0.01$) and *Hallea ledermannii* ($p < 0.01$) compared to the non-diabetic control. Nevertheless, significant decreases ($p < 0.001$) in blood glucose were observed in animals treated with glibenclamide and extracts of EAs and EAH compared to untreated diabetic controls (Figure 4C). Figure 4D (J₅₆) shows a significant ($p < 0.05$) increase in blood glucose levels in animals treated with EAs₂₀₀ and *Hallea ledermannii* (200 and 400 mg/kg bw) relative to the nondiabetic control. After 56 days of treatment, glycaemia in rats treated with glibenclamide and EAs₁₀₀ decreased and became statistically similar ($p > 0.05$) to that of the nondiabetic control. Blood glucose levels of all treated rat lots were significantly ($p < 0.001$) lower than the untreated diabetic control. After 91 days of daily administration of the test substances, we

observed a nonsignificant change in glycemia of the glibenclamide treated rats and EAAs and EAHL compared to non-diabetic controls (Figure 4E). Blood glucose levels in untreated diabetic rats remained high ($p < 0.001$) compared with non-diabetic controls.

Assessment of the effects of *Annona senegalensis* and *Hallea ledermannii* on hepatic glucose storage in diabetic rats

After 13 weeks (J91) of treatment, the glucose storage test in the liver of diabetic rats was performed (Figure 5). This study shows in diabetic animals treated with glibenclamide and plant extracts, a statistically similar amount of glucose ($p > 0.05$) to that of the non-diabetic control. This test reveals that the liver of untreated diabetic rats has a low ($p < 0.05$) glucose retention capacity relative to the non-diabetic control. The glucose level stored in the liver of diabetic rats treated with glibenclamide and EAHL200 is higher ($p < 0.05$) than the untreated diabetic rat.

Effects of *Annona senegalensis* and *Hallea ledermannii* on glycated hemoglobin content in diabetic rats

The glycated hemoglobin content of diabetic rats after 91 days of daily treatment is shown in Figure 6 which shows the influence of glibenclamide and extracts of both plants. This parameter does not show significant variations ($p > 0.05$) in animals treated with glibenclamide and extracts of *Annona senegalensis* and *Hallea ledermannii* compared to the non-diabetic control. In untreated diabetic animals, the glycated hemoglobin content increased significantly ($p < 0.05$) compared to the non-diabetic control. This parameter decreased by 37.5% ($p < 0.05$) in diabetic rats treated with EAAs100 compared to untreated diabetic animals.

Influence of *Annona senegalensis* and *Hallea ledermannii* extracts on lipid profile in diabetic rats

Table 1 shows the effects of the treatments on the lipid profile of diabetic rats during the experimental period. The results obtained show that the total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol levels in untreated diabetic rats as well as in those treated with glibenclamide and extracts did not vary significantly ($p > 0.05$) compared to the non-diabetic control. The LDL cholesterol (LDL-c) value in EAAs200-treated animals significantly decreased ($p < 0.05$) compared

to untreated diabetic controls after four weeks of daily dosing.

Macroscopic examination analysis of the effects of *Annona senegalensis* and *Hallea ledermannii* on the pancreas

In macroscopic examination, no changes in pancreas were observed in the diabetic rats compared to the non- control, after 13 weeks (J91) of treatment.

The effects of *Annona senegalensis* and *Hallea ledermannii* on the relative weight of the pancreas were evaluated on D28 and D91. All values did not change significantly ($p > 0.05$) in all diabetic groups (treated or untreated) compared to the nondiabetic control (Table 2).

Discussion

The determination of polyphenols and total flavonoids in the aqueous extracts of *Annona senegalensis* and *Hallea ledermannii* shows that *Annona senegalensis* extract contains a higher amount of polyphenols and flavonoids than *Hallea ledermannii*.

The anti-radical test materialized by the percentage inhibition of the DPPH radical as a function of quercetin, EAAs and EAHL concentrations, shows that these substances reduce this free radical and have dose-dependent effects. However, this study revealed that quercetin has a higher anti-radical activity than EAAs and EAHL. These results are different from those of Neha et al. (30) who showed that leaf and stem extracts of *tectona grandis* had greater antiradical activity than quercetin. Protonic anti-radical action is an oxidation mechanism. DPPH possesses a protonic free radical and exhibits a characteristic absorption induced by antiradical substances (31). This suggests that the antioxidant activity of the leaf extract of *Annona senegalensis* and *Hallea ledermannii* is due to its ability to give protons, which shows a correlation between the content of flavonoids and polyphenols and the antioxidant activity of the compounds (32).

In alloxan-induced diabetic rats, the daily administration of extracts of *Hallea ledermannii*, (EAH400), a weight loss was passed between two weeks (J14) of treatment, compared to the normal control value. After four (4) weeks of treatment (D28), body weight decreases were observed in rats treated with EAAs and EAHL. This decrease was very

high in untreated diabetic controls compared to the nondiabetic control. These results are contrary to those reported by Tastekin et al. (33) who showed, in treated diabetic rats in 15 days with an aqueous extract of *Artemisia herba alba* at the daily dose of 390 mg/kg, an increase of body weight. This is the same for Viswanathan (34), who obtained weight gain in rats treated with the aqueous extract of *Mimosa pudica* after four weeks of treatment.

After eight (8) weeks (D56) of daily administration of the different substances, body weight increased in rats treated with glibenclamide and those treated with EAs100 compared to the untreated diabetic control. These results are similar to those of Medjdoub et al. (35) showing that the change in weight of diabetics treated with FA3 fraction of *Zygophyllum geslini* at 100 mg/kg is corrected at the 4th week of treatment. However, a decrease was observed in untreated diabetic rats and those treated with EAH1200 and EAH1400 compared to the normal control. This decrease in weight is in agreement with the results obtained by some authors who showed a regression of this parameter in rats made diabetic by the administration of streptozotocin (36, 37).

Over a 13-week (J91) treatment period, animal weights decreased in untreated diabetic controls and in those treated with EAH1200 and EAH1400 compared to the non-diabetic control. In animals treated with Gli10, EAs100 and EAs200, the body weight has increased, compared to untreated diabetic controls.

The anti-diabetic effects of *Annona senegalensis* (EAs) and *Hallea ledermannii* (EAH) in diabetic rats were tested at the beginning (D0) of the test and after two weeks (D14), four weeks (D28), eight weeks (D56) and thirteen weeks (J91) of treatment.

From day 0 to day 14, the blood glucose levels of diabetic rats and those treated with EAs200 and EAH are very significantly elevated compared with that of the non-diabetic control rats. However, the decreases in blood glucose in the lots treated with glibenclamide and EAs100, were observed compared to the untreated diabetic control. Our results corroborate those of Ahmed et al. (38) who showed that a 14-day treatment with the methanolic extract of *Vinca rosea* with a daily dose of 500 mg/kg bw reduced hyperglycemia in diabetic rats.

At day 28, the blood glucose level remains high in diabetic animals (treated or not) compared with the non-diabetic control. Nevertheless, significant decreases in blood glucose were observed in animals treated with glibenclamide and EAs and EAH compared to untreated diabetic controls. These results therefore confirm the first conclusions of Al-Shamaony et al. (39) who found that administration of the aqueous extract of *Artemisia herba alba* Asso (390 mg/kg bw) caused a highly significant decrease in blood glucose levels in the diabetic rat, just after four weeks of treatment.

After 56 days of treatment, glycaemia in rats treated with glibenclamide and EAs decreased and became statistically similar to that of the nondiabetic control. Blood glucose levels of all treated rat lots were significantly lower than the untreated diabetic control. These results have been confirmed in the work of Medjdoub et al. (35) showing that glimepiride 0.1 mg/kg and fraction FA3 *Zygophyllum geslini* 100 mg / kg cause a significant reduction in blood glucose over four weeks of experimentation.

Thirteen (13) weeks of daily administration of the substances allowed us to observe a nonsignificant decrease in blood glucose levels in rats treated with glibenclamide, EAs and EAH compared with non-diabetic controls. In the present study, we found that extracts of *Annona senegalensis* and *Hallea ledermannii* resulted in lower blood sugar. These results are similar to those of Daisy and Jeeva Kani (40) who showed that daily administration over a 90-day period of hexanic, ethyl acetic and methanolic extracts of *Cassia auriculata* caused a decrease in blood glucose levels in rats. diabetics. These authors showed that the antidiabetic activity of this plant was related to its effect on the amount of insulin which increases in treated diabetic rats.

The glucose storage test in the liver of diabetic rats shows that in control diabetic rats, the hepatic glucose level is reduced compared with the non-diabetic control. This decrease observed in these rats is explained by an alteration of the secretion of insulin after the administration of the alloxan monohydrate. Indeed, Shetti et al. (41) showed that alloxan impairs insulin secretion, which leads to an inhibition of glucokinase activity. When the diabetic rats are treated with EAs and EAH, after 91 days, the hepatic glucose level increases and becomes

substantially identical to that of the normal control rats. These results show that EAAs and EAHL promote the storage of glucose in the liver. Other similar results have been reported by Kebièche (42) and Ladouari (43). These authors respectively showed that the extracts of *Ranunculus repens* (Ranunculaceae) and *Zygophyllum album* (Zygophyllaceae), favor the storage of glucose in the liver. The effect of EAAs or EAHL on hepatic glucose storage is similar to that of glibenclamide. It may therefore be suggested that, just like glibenclamide, the mechanism of action of EAAs or EAHL is through its direct action on Glut 2 (glucose transporter in the cell) receptors or stimulation of residual pancreatic β -cells for allow the storage of glucose.

The glycated hemoglobin (HbA_{1c}) content of diabetic rats after 91 days, shows the influence of glibenclamide and extracts of *Annona senegalensis* and *Hallea ledermannii* on blood glucose. This parameter does not show significant variations in the animals treated with glibenclamide and the two plant extracts, compared with the non-diabetic control. The glycated hemoglobin decreased in diabetic rats treated with EAAs100 compared to control diabetic animals. Similar results were obtained by Olowu et al. (44) and Bassem et al. (45), who demonstrated the reduction of HbA_{1c} in diabetic rats treated respectively with natural honey for eight (8) weeks and with Zamzam water.

The effects of *Annona senegalensis* and *Hallea ledermannii* extracts on the lipid profile in diabetic rats were determined. The results show that only the LDL cholesterol (LDL-c) value in EAAs200 treated animals (200 mg/kg bw) significantly decreased compared to untreated diabetic controls after four weeks daily administration. In contrast to these data, the lipid profile remains unchanged after thirteen (13) weeks of treatment. These results are similar to those of Thomson et al. (46) which indicates that garlic normalizes the lipid profile of animals. This cholesterol-lowering activity of EAAs200 is probably due to certain constituents that may act as inhibitors of certain enzymes such as hydroxy methyl glutaryl-CoA "HMG CoA" reductase, key enzyme of cholesterol synthesis (47, 48).

The effects of *Annona senegalensis* and *Hallea ledermannii* in alloxan-induced diabetic rats, seem be due to the presence of some bioactive

compounds as flavonoids, saponosides, triterpenes and tannins which influence pancreatic β cells and stimulate the secretion of insulin through their antioxidant activities (49, 50).

In conclusion, it is demonstrated that the crude aqueous extrats of leaves of *Annona senegalensis* and *Hallea ledermannii* possesses the antidiabetic properties in alloxan-induced diabetic rats which seem due to their antioxidant activities through the bioactive compounds as flavonoids, saponosides, triterpenes and tannins. Therefore, these plants are good candidates for further studies with their fractions .

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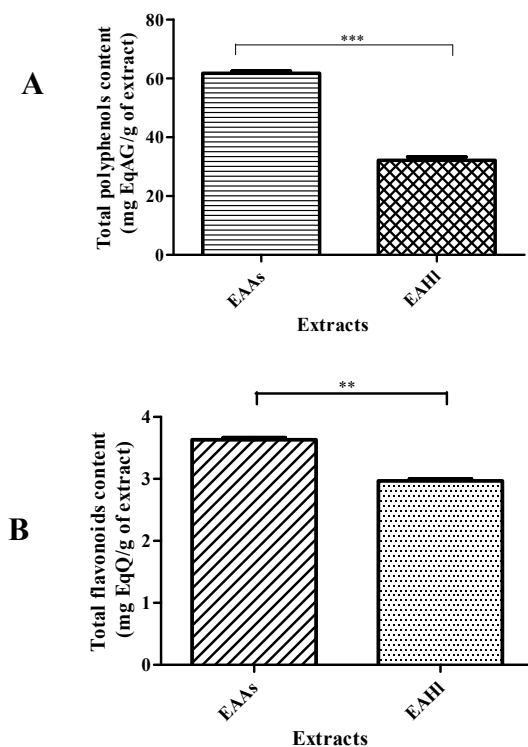


Figure 1: Content of polyphenols (A) and flavonoids (B) of aqueous extracts of *Annona senegalensis* and *Hallea ledermannii*. The data are expressed as mean \pm SEM, (** $p < 0.01$, *** $p < 0.001$, $n = 3$).

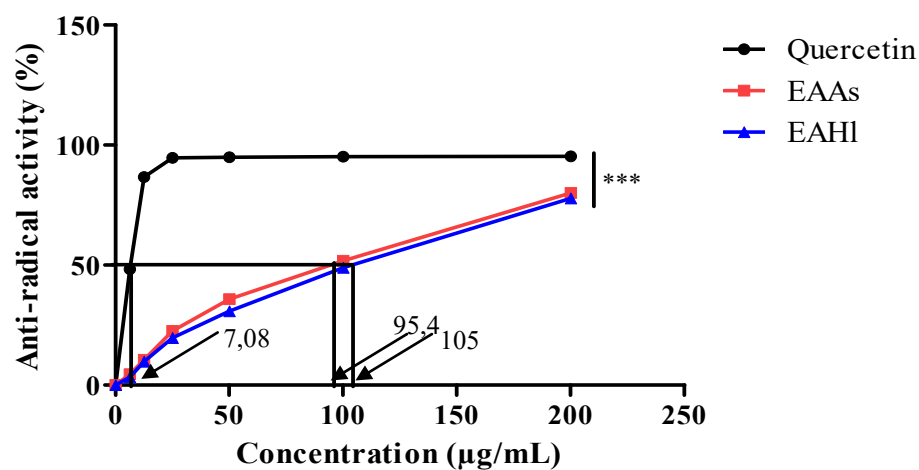


Figure 2: Percentage of inhibition of the DPPH radical as a function of quercetin concentrations and aqueous extracts of *Annona senegalensis* and *Hallea ledermannii*. The reference substance has a low IC₅₀ compared to both extracts. The data are presented as mean \pm SEM, n = 3 (***) p < 0.001).

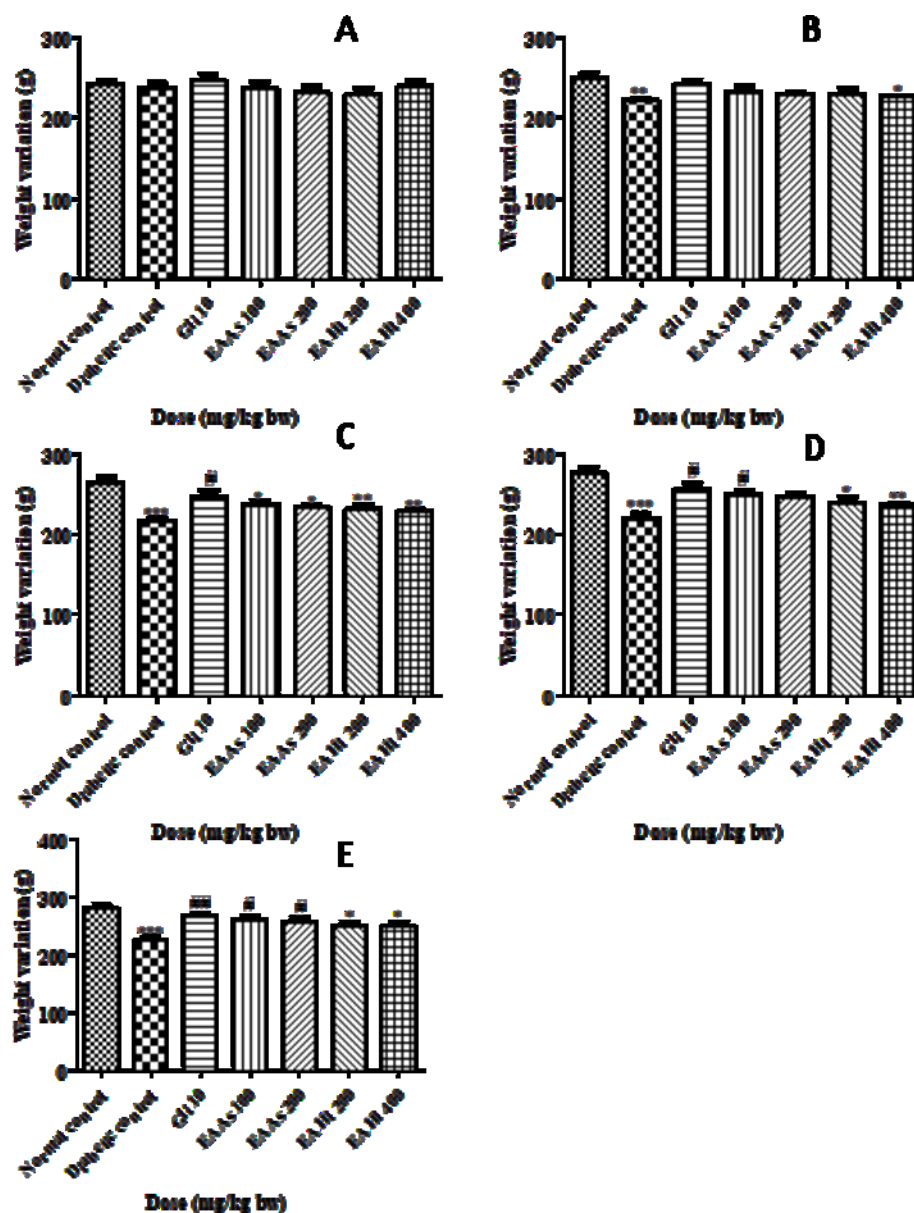


Figure 3: Evaluation of the effects of Glibenclamide, *Annona senegalensis* and *Hallea ledermannii* on the weight of diabetic rats. J0 (A), J14 (B), J28 (C), J56 (D), J91 (E). The results are presented on average \pm SEM, $n = 6$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to nondiabetic control, # $p < 0.05$; ## $p < 0.01$ compared to untreated diabetic control.

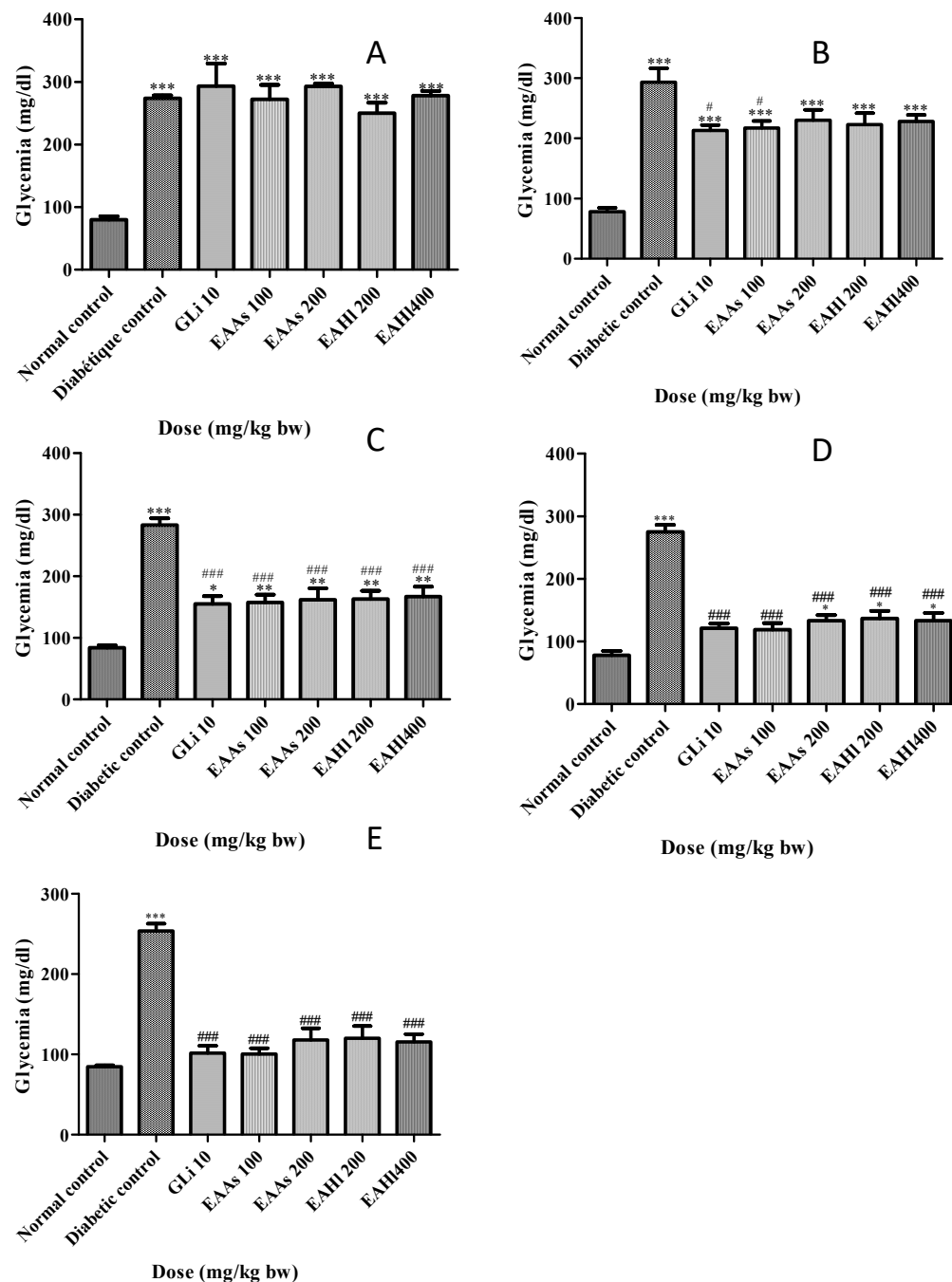


Figure 4: Effects of EAAs, EAH and glibenclamide extracts on glycemia in diabetic rats. J0 (A), J14 (B), J28 (C), J56 (D), J91 (E). The results are presented on average \pm SEM, $n = 6$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to nondiabetic control, # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ compared to untreated diabetic control.

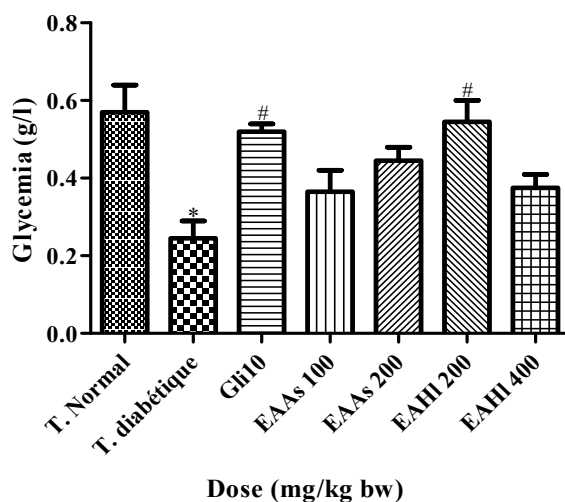


Figure 5: Influence of EAAs, EAHI and glibenclamide extracts on hepatic glucose storage in rats after 13 weeks of treatment. The results are presented as mean \pm SEM, $n = 6$; * $p < 0.05$ compared to non-diabetic control, # $p < 0.05$ compared to untreated diabetic control.

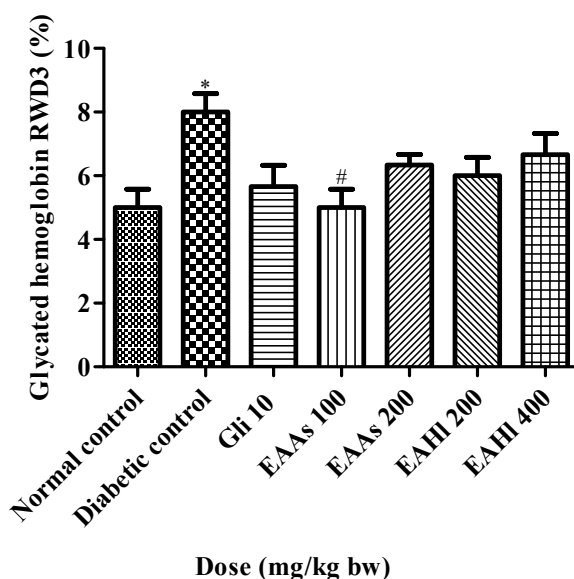


Figure 6: Influence of EAAs, EAHI and glibenclamide extracts on glycated hemoglobin content in rats after 13 weeks of treatment. The results are presented as mean \pm SEM, $n = 6$; * $p < 0.05$ compared to non-diabetic control, # $p < 0.05$ compared to untreated diabetic control.

Table I : Influence of *Annona senegalensis* and *Hallea ledermannii* extracts on lipid profile in diabetic rats

	Normal control	Diabetic control	Gli10	EAA5100	EAA5200	EAL1200	EAL1400
2 Weeks							
Chol T (g/L)	1,87±0,03	2,03±0,08	1,83±0,03	1,98±0,11	1,81±0,02	1,82±0,05	1,88±0,11
Trigly (g/L)	0,75±0,05	0,75±0,07	0,77±0,04	0,73±0,05	1,01±0,07	0,76±0,1	0,78±0,05
HDL-Chol (g/L)	0,44±0,01	0,43±0,02	0,43±0,02	0,46±0,02	0,44±0,02	0,42±0,02	0,47±0,02
LDL-Chol (g/L)	1,27±0,02	1,39±0,03	1,28±0,02	1,28±0,03	1,2±0,03	1,25±0,02	1,29±0,1
4 Weeks							
Chol T (g/L)	1,88±0,03	1,99±0,05	1,84±0,03	1,88±0,05	1,80±0,01	1,81±0,05	1,87±0,10
Trigly (g/L)	0,76±0,05	0,75±0,07	0,78±0,04	0,72±0,05	1±0,07	0,74±0,10	0,75±0,06
HDL-Chol (g/L)	0,45±0,01	0,44±0,02	0,42±0,02	0,46±0,02	0,42±0,02	0,41±0,02	0,46±0,02
LDL-Chol (g/L)	1,27±0,02	1,39±0,03	1,27±0,02	1,28±0,03	1,18±0,03 [#]	1,24±0,02	1,27±0,08
8 Weeks							
Chol T (g/L)	1,82±0,08	1,85±0,17	1,94±0,17	1,82±0,17	2,09±0,23	1,85±0,05	1,72±0,16
Trigly (g/L)	0,79±0,06	0,75±0,05	0,83±0,06	0,76±0,14	0,82±0,1	0,84±0,03	0,60±0,16
HDL-Chol (g/L)	0,44±0,02	0,42±0,04	0,44±0,03	0,39±0,03	0,49±0,05	0,46±0,03	0,39±0,08
LDL-Chol (g/L)	1,22±0,07	1,28±0,13	1,33±0,15	1,28±0,12	1,43±0,16	1,22±0,03	1,21±0,05
13 Weeks							
Chol T (g/L)	0,91±0,01	0,86±0,02	0,84±0,03	0,85±0,08	0,74±0,08	0,87±0,03	0,97±0,09
Trigly (g/L)	0,49±0,01	0,37±0,06	0,34±0,06	0,49±0,12	0,63±0,01	0,71±0,05	0,63±0,05
HDL-Chol (g/L)	0,30±0,02	0,28±0,02	0,30±0,04	0,30±0,01	0,22±0,01	0,29±0,02	0,25±0,05
LDL-Chol (g/L)	0,44±0,03	0,48±0,04	0,48±0,02	0,46±0,06	0,40±0,08	0,44±0,05	0,59±0,03

Data are presented as mean ± SEM, n = 6; [#]p<0,05 compared to diabetic control.

Table 2: Influence of *Annona senegalensis* and *Hallea ledermannii* on the relative weight of the pancreas in diabetic rats after 4 and 13 weeks of treatment

	Pancreas	
	4 weeks of treatment	13 weeks of treatment
Normal control	0,39±0,08	0,42±0,09
Diabetic control	0,24±0,01	0,35±0,03
Gli 10 (10 mg/kg bw)	0,30±0,01	0,25±0,01
EAs 100 (100 mg/kg bw)	0,26±0,01	0,24±0,01
EAs 200 (200 mg/kg bw)	0,29±0,01	0,25±0,03
EAHI 200 (200 mg/kg bw)	0,37±0,04	0,26±0,05
EAHI 400 (400 mg/kg bw)	0,27±0,04	0,25±0,01

Data are presented as mean ± SEM