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Antioxidant Potentials of *Vernonia amygdalina* (Asteraceae), Antidiabetic Plant, "In Vitro" and "In Vivo" in Healthy Rats and Diabetic Rats

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ABSTRACT

Some herbal remedies are said to have antioxidant activity, reducing oxidative stress in cells and are therefore useful in the treatment of many diseases such as diabetes. This study consisted of the evaluation of the antioxidant activity *in vitro* and *in vivo* of an aqueous extract of *Vernonia amygdalina* (EAVa) in healthy rats and diabetic rats. The antioxidant power of EAVa, evaluated *in vitro* by assaying the anti-free radical activity by the 2,2-diphenyl-1-picrylhydrazyl test, shows that this extract (IC_{50} = 26.07 µg/mL), just like gallic acid (IC_{50} = 2.13 µg/mL), reduces this free radical, and therefore has an antioxidant power. The study of antioxidant activity *in vivo* on rats shows that EAVa, at a dose of 1200 mg/kg BW, strengthens the antioxidant balance in healthy animals and decreases the state of oxidative stress induced by the injection. streptozotocin (STZ). In fact, the injection of STZ disrupts the oxidative defense system in rats. In pathological changes caused by the presence of oxygen radicals, EAVa causes depletion of hepatic glutathione levels and increased levels of liver malondialdehyde, catalase and superoxide dismutase. EAVa thus maintains the balance of the antioxidant balance of liver cells, which allows it to be used against diseases associated with oxidative stress, and therefore the pathological state of diabetes. This antioxidant power of the aqueous extract of *Vernonia amygdalina* would justify the use of this plant in the prevention and treatment of diabetes and associated pathologies.

Keywords: Diabetes, antioxidant, *Vernonia amygdalina*, hepatic glutathione, catalase, malondialdehyde, superoxide dismutase.

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INTRODUCTION

An antioxidant is a more or less complex chemical species that decreases oxidative stress in the body. Oxidative stress is an attack on the constituents of the cell due to reactive oxygenated species. It corresponds to an imbalance between pro-oxidant molecules (reactive oxygen species) and antioxidant defense molecules at the cellular level[1]. Antioxidants are able to neutralize free radicals circulating in the body and thus fight against oxidative stress. This oxidative stress is notably at the origin of most of the inflammatory processes associated with many chronic pathologies such as diabetes.

Diabetes mellitus is accompanied by oxidative stress and the production of free radicals by various mechanisms. Several studies report an association between diabetes and oxidative stress. Many herbal remedies contain antioxidant compounds which protect cells from the deleterious effects of reactive oxygen species[2] and, for such properties, are useful in the treatment of many diseases such as diabetes.

Vernonia amygdalina (Asteraceae) is a medicinal plant used in traditional medicine in Ivory Coast to treat diabetes. The aim of this study is to evaluate the antioxidant activity *in vitro* and *in vivo* of an aqueous extract of *Vernonia amygdalina* (EAVa) in healthy rats and diabetic rats.

MATERIALS AND METHOD

Animal material

Rats, *Rattus norvegicus* (Murideae), female and male, of Wistar strain, from the animal facility of the Ecole Normale Supérieur (ENS) (Abidjan, Ivory Coast), weighing between 150 g and 250 g, are used for the evaluation of antioxidant activity *in vivo*.

These animals are reared separately in cages at a temperature of 21 ± 2° C, with a humidity of 55 to 60 % and a photoperiod of 12 hours of light and 12 hours of darkness. These animals are given water and a feed composed of granules (Ivograins®) at will. All the experimental protocols are conducted in accordance with the European directive of November 24, 1986 (86/609/EEC) and the decree of April 19, 1988[3] relating to the use of experimental animals in research.

Plant material

The plant material consists of dry leaves of *Vernonia amygdalina* Del. (Asteraceae). These leaves are collected in the morning, in March 2018, in Ivory Coast, in Dokanou (Koun-Fao department, Gontougo region, Geographical coordinates: Latitude: N 7° 57'; Longitude: E - 3° 30'; Altitude: 207 meters). This plant has been identified and authenticated at the National Floristic Center (CNF) of the University Félix Houphouët-Boigny (UFHB), in comparison with the herbarium found there under the number: AKE Assi L. 11694 Sakré, 27 March 1972.

Substances

In this study gallic acid, glibenclamide, hydrogen chloride, streptozotocin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and quercetin are used.

Methods

Preparation of the aqueous extract of *Vernonia amygdalina* (Asteraceae)

For the preparation of the aqueous extract of *Vernonia amygdalina* (Asteraceae), the leaves of this plant are harvested and then rinsed with distilled water, before being dried away from sunlight and at room temperature, at the Laboratory of Biology and Health of the UFR Biosciences, at the UFHB. The dry leaves are then finely ground. One hundred grams (100 g) of powdered dry leaves of *Vernonia amygdalina* are brought to the boil for 15 min at 100º C in 2 liters of distilled water[4]. The decocté obtained is filtered twice through cotton wool, then once through Whatman No. 2 filter paper. The filtrate collected is dried in an oven (Vacutherm Vacuum Oven, France) at 40° C for 72 hours. After drying, the dry pellet is recovered and reduced to powder. The powder obtained is the aqueous extract of *Vernonia amygdalina* (EAVa).

DPPH (2,2-Diphenyl-1-picrylhydrazyl) test

A concentration range of EAVa from 0 to 200 µg/mL or of quercetin (reference antioxidant) is prepared in an ethanol/water mixture (70/30) (v/v). A volume of 100 µL of this solution is mixed with 3.9 mL of 70 µM DPPH, prepared in methanol. After homogenization, the mixture is incubated at room temperature (25° C) protected from light. After 15 minutes of incubation, the absorbance is read at λ = 517 nm against a "blank" which contains only methanol.

The 50 % inhibitory concentrations (IC_{50}), which are the concentrations of extract or quercetin responsible for 50 % inhibition of DPPH radicals, are determined graphically on the curve representing the percentage of inhibition of DPPH as a function of the concentrations of the extract or quercetin. Each result constitutes the average of the three values obtained (n = 3).

***In vivo* study of antioxidant activity**

Induction of diabetes

To confirm that the rats are not normally diabetic, blood samples are taken to measure their fasting glucose levels before the subcutaneous injection of streptozotocin.

Wistar rats, with an average weight between 150 and 250 g, are made diabetic by subcutaneous injection of nicotinamide (210 mg/kg BW) followed by streptozotocin (STZ) at 60 mg/kg BW[5] STZ, freshly prepared in a citrate buffer solution (0.1 M ; pH = 4.5), is capable of inducing fatal hypoglycemia resulting from massive pancreatic secretion of insulin. Thus, to prevent this fatal

effect after the administration of streptozotocin, the rats are watered with a 5 % glucose solution overnight.

After the injection of streptozotocin, the rats are screened by assaying their blood sugar. Those with a permanent blood sugar level above 2.5 g/L (diabetic rats) [6]. Only diabetic rats (permanent diabetes) are retained for the experiments on diabetes. They are transferred to individual metabolic cages.

Study of the effects of the aqueous extract of *Vernonia amygdalina* (EAVa) on the antioxidant activity *in vivo* of diabetic rats

To study the antioxidant activity *in vivo*, 100 rats are divided into 5 groups of 20 rats.

Batch I: the rats in this batch are healthy (normoglycemic). They are given distilled water, which also serves as non-diabetic controls.

Batch II: the rats in this batch are normoglycemic (non-diabetic). They receive EAVa at a dose of 1200 mg/kg BW.

Batch III: the rats in this batch are diabetic and receive distilled water. This batch serves as a diabetic control (untreated diabetics).

Batch IV: the rats in this batch are those made diabetic and treated with 200 mg/kg BW of gallic acid (AG).

Batch V: the rats in this batch are those made diabetic and treated with 1200 mg/kg BW of EAVa.

The rats are treated for 28 days. At the start of the experiment (1st day) and every 7 days, 4 rats per batch are sacrificed and their livers recovered in order to measure the activity of hepatic antioxidants. Thus, hepatic glutathione activity, malondialdehyde activity, catalase enzymatic activity and superoxide dismutase enzymatic activity are assayed.

Determination of hepatic glutathione activity

For the hepatic glutathione (GSH) assay, 1 g of rat liver is taken and ground in 3 volumes of 5 % trichloroacetate (TCA) in a mortar, then centrifuged at 2000 rpm for 15 min using a centrifuge (Alliance Bio Expertises, Belgium). Fifty microliter (50 µL) of the supernatant are diluted in 10 mL of phosphate buffer (0.1 M ; pH = 8). To 3 mL of the dilution mixture, 20 µL of 0.01 M Ellman's reagent are added. The absorbance is read, using a spectrophotometer (BIOLABO Diagnostics, France), at $\lambda = 412$ nm, against a "blank" prepared under the same conditions with 5 % TCA. The concentrations are expressed in nmol/g of liver.

Determination of malondialdehyde activity

For the determination of malondialdehyde (MDA), 1 g of rat liver is ground in a mortar with 3 mL of KCl solution (1.15 M), then 0.5 mL of the ground material, 0.5 mL of trichloroacetic acid 20 %

and 1 mL of 0.67 % thiobarbituric acid (TBA) are mixed. The homogeneous mixture is heated at 100° C. for 15 min. It is cooled, then added to 4 mL of n-butanol. After centrifugation at 3000 rpm for 15 min, the optical density of the supernatant is determined with a spectrophotometer at λ = 530 nm. The amount of MDA in the sample is expressed in nmol/g of liver.

Determination of the enzymatic activity of catalase

To measure the enzymatic activity of catalase (CAT), an enzyme fraction is prepared[7]. Two grams (2 g) of rat liver are cut and ground in a mortar with 3 volumes of phosphate buffers (0.1 M ; pH = 7.4) containing KCL (1.17 %). The ground material is centrifuged at 2000 rpm for 15 min at 4° C. The supernatant obtained is also centrifuged at 9600 rpm for 30 min at 4° C. The supernatant thus obtained is used for the evaluation of catalase activity. For this, 1 mL of phosphate buffer (KH_2PO_4; 0.1 M ; pH = 7.2), 0.975 mL of freshly prepared H_2O_2 (0.091 M) and 0.025 mL of the enzyme source (the cytosol) are mixed. The absorbance is read with a spectrophotometer at λ = 240 nm every 30 seconds, for 2 min. Enzyme activity is expressed in international units per minute per gram of liver (UI/min/g of liver).

Determination of the enzymatic activity of superoxide dismutase

A mixture, composed of 5 µL of cytosol and 300 µL of each of the following solutions: Tetrazolium nitroblue (NBT), methionine and riboflavin (pH = 7.8), is exposed to the light of a 15 W lamp for 10 min to induce the photo-reaction of riboflavin and oxygen (O_2). The optical density is read with a spectrophotometer at λ = 560 nm. Enzyme activity is expressed in UI/mg liver protein.

Statistical and graphical analyzes

Statistical analyzes of values and graphical representations of data are performed using *GraphPad Prism 8* software (San Diego, California, USA). The statistical difference between the results is determined by analysis of variance (ANOVA), followed by the Tukey-Kramer multiple comparison test, with a significance level $p < 0.05$. The results are expressed as the mean ± error of the mean (ESM).

RESULTS AND DISCUSSION

Determination of the anti-free radical activity of the aqueous extract of *Vernonia amygdalina* (EAVa) by the DPPH test

The results of the measurement of the absorbance of the free radical 2,2-Diphenyl-l-Picrylhydrazyl (DPPH) under the action of EAVa or quercetin (reference antioxidant), allows to draw the curves of variation of inhibition of this free radical as a function of the concentrations of these substances (**Figure 1**). This figure shows that the antioxidant activities of α-EAVa or quercetin, expressed as

a percentage inhibition of the DPPH radical, are dependent on the concentration of these substances. The anti-free radical activity of EAVa, determined by the percentage inhibition of DPPH, gives a 50 % inhibitory concentration (IC_{50}) of 26.07 µg/mL; that of quercetin is 2.13 µg/mL. The correlation coefficient (R^2) is 0.9784 for EAVa and 0.9228 for quercetin.

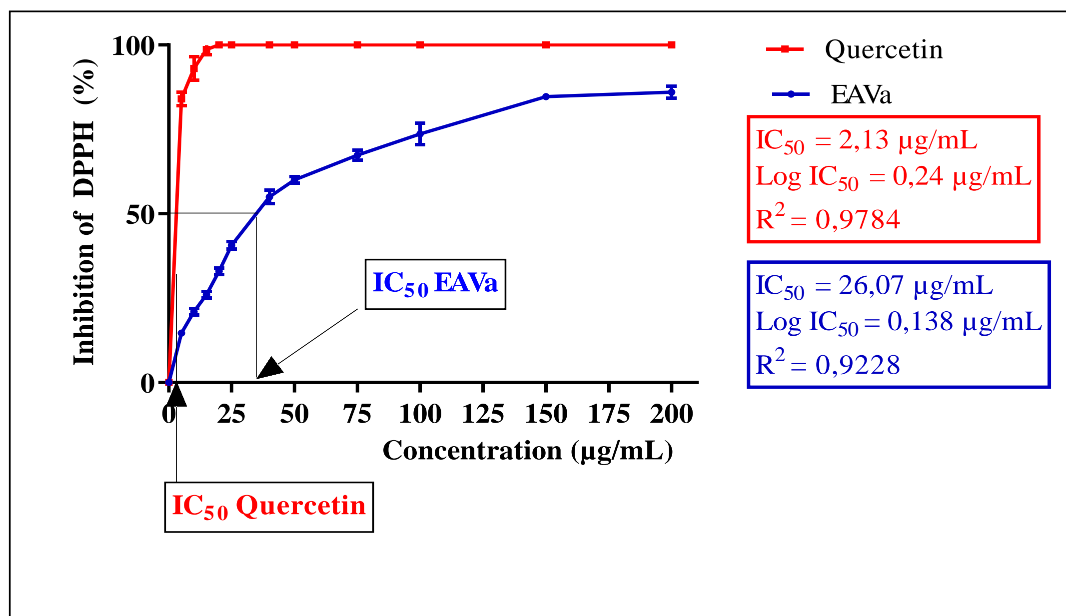

Figure 1: Percentage inhibition of the DPPH radical by the aqueous extract of *Vernonia amygdalina* (EAVa) or quercetin

Determination of the antioxidant power *in vivo* of the aqueous extract of *Vernonia amygdalina* (EAVa) in diabetic rats

Effects of the aqueous extract of *Vernonia amygdalina* (EAVa) and gallic acid on hepatic glutathione activity in diabetic rats

In non-diabetic control rats, the hepatic glutathione (GSH) level at the start of the experiment (1st day) was 6.32 ± 0.04 nmol/mg protein. These rates do not vary significantly ($p > 0.05$) during the 28 days of experimentation when the rats are not treated (control groups) (**Figure 2**). On the other hand, in diabetic control rats, the level of GSH is low. It is 0.53 ± 0.07 nmol/mg of proteins on 1st day; a very significant decrease ($p < 0.001$) in the activity of hepatic antioxidant enzymes of 91.61 % compared to that of non-diabetic control rats, when the rats are made diabetic by injection of streptozotocin (STZ).

When non-diabetic rats received orally EAVa at a dose of 1200 mg/kg BW, the rate of reduction of GSH increased insignificantly ($p > 0.05$) until the 28th day of treatment, with a value of 6.46 ± 0.01 nmol/mg protein; ie an increase of 2.22 % compared to that of the non-diabetic control rats (on 1st day).

When rats made diabetic are treated with EAVa (1200 mg/kg BW) or gallic acid (200 mg/kg BW), hepatic glutathione redox levels gradually increase. Thus, on the 28th day of treatment of the rats, the reduced GSH levels are 2.14 ± 0.03 nmol/mg and 3.18 ± 0.01 nmol/mg of proteins in the rats given respectively the EAVa or gallic acid; ie respective increases in hepatic antioxidant enzyme activity of 303.77 % and 500 % ($p < 0.001$), compared to that of diabetic control rats.

The results of this series of experiments show that EAVa and AG protect the body against oxygen free radicals. However, compared to diabetic control rats, the activity of hepatic antioxidant enzymes in diabetic rats treated with these two substances was lower, with a greater effect on AG.

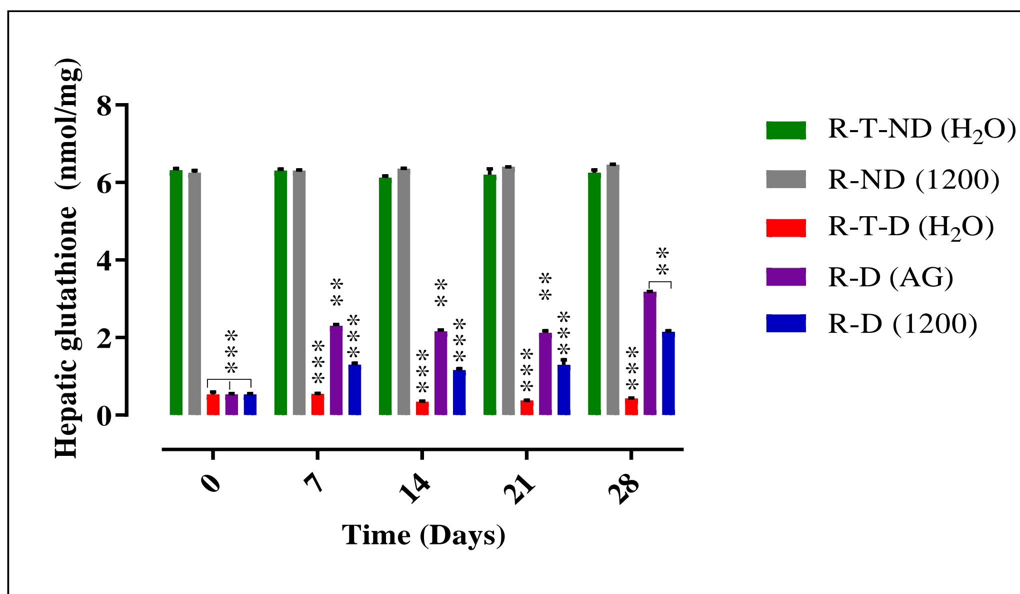

Figure 2: Change in reduced hepatic glutathione level in treated diabetic rats with aqueous extract of *Vernonia amygdalina* (EAVa) or gallic acid

*n = 4; ** p <0.01; *** p < 0.001 compared to non-diabetic controls*

R-T-ND (H_2O): Non-diabetic control rats

R-ND (1200) : Non-diabetic rats treated with 1200 mg/kg BW of EAVa

R-D (H_2O) : Diabetic control rats

R-D (AG) : Diabetic rats treated with 200 mg/kg BW of gallic acid (AG)

R-D (1200) : Diabetic rats treated with 1200 mg/kg BW of EAVa

Effects of the aqueous extract of *Vernonia amygdalina* (EAVa) and gallic acid on malondialdehyde activity in diabetic rats

Injection of STZ results in a very significant ($p < 0.001$) increase in the level of malondialdehyde (MDA). Indeed, before the animal treatments (1st day), the level of MDA, which is 0.15 ± 0.02 nmol/mg of proteins in non-diabetic control rats, increases to 0.55 ± 0.01 nmol/mg of protein when

rats are made diabetic; i.e. an increase of 266.67 % (**Figure 3**). These values remain substantially identical ($p > 0.05$) in the diabetic control rats until the 28th day of experimentation.

Administration of EAVa at a dose of 1200 mg/kg BW to non-diabetic rats induces a non-significant decrease ($p > 0.05$) in the level of MDA which, until the 28th day of treatment, decreases by 13.33 % in these healthy animals, with a rate of 0.13 ± 0.01 nmol/mg of proteins, against 0.15 ± 0.02 nmol/mg of proteins on 1st day (non-diabetic control).

When diabetic rats are treated with gallic acid (AG) at 200 mg/kg BW, on day 14, the level of MDA increases to 0.42 ± 0.01 nmol/mg protein; ie a significant decrease ($p < 0.01$) of 23.64 % compared to that of the diabetic control rats, and this rate remains substantially identical ($p > 0.05$) until the 28th day of experimentation.

In diabetic rats treated with EAVa at a dose of 1200 mg/kg BW, the reduction in MDA level appears significant ($p < 0.01$) on day 14. Thus, on the 28th day of treatment, a significant reduction ($p < 0.001$) of 36.36 % is obtained with an MDA level of 0.34 ± 0.02 nmol/mg of protein which is maintained ($p > 0.05$) until the 28th day of treatment.

EAVa and gallic acid therefore significantly reduce the increase in MDA that occurs following induction of diabetes in rats, with a greater effect in animals treated with EAVa. However, on day 28 of treatment of diabetic rats with EAVa or AG, MDA levels increased by 118.53 % and 180.50 %, respectively, compared to that of non-diabetic control rats. Also, EAVa did not have a significant effect on the level of MDA in healthy rats.

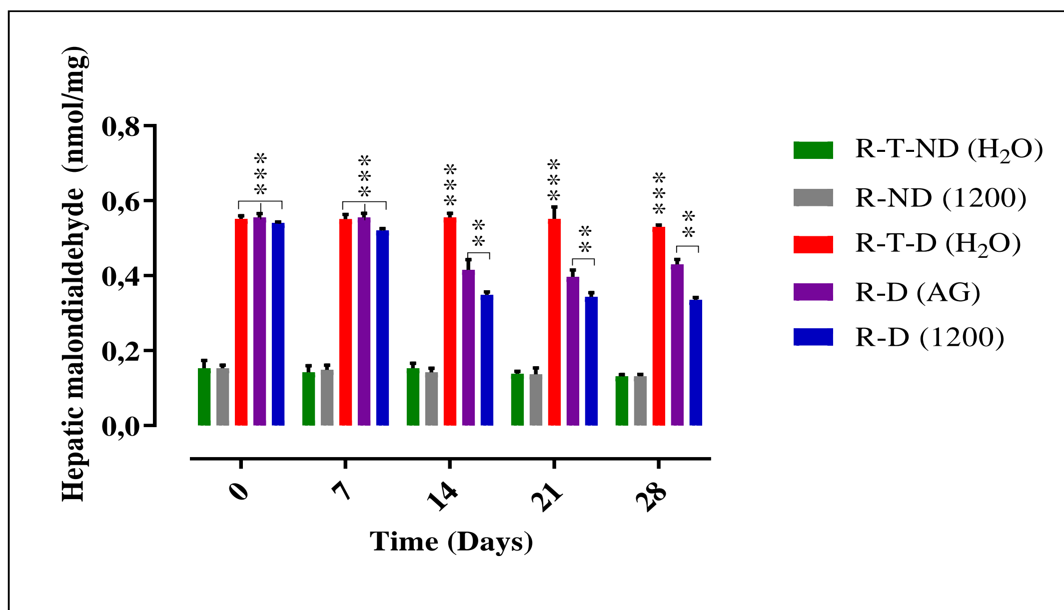

Figure 3: Change in hepatic malondialdehyde level in treated diabetic rats with aqueous extract of *Vernonia amygdalina* (EAVa) or gallic acid

*n = 4; ** p <0.01; *** p <0.001 compared to non-diabetic controls*

R-T-ND (H_2O): Non-diabetic control rats

R-ND (1200) : Non-diabetic rats treated with 1200 mg/kg BW of EAVa

R-D (H_2O) : Diabetic control rats

R-D (AG) : Diabetic rats treated with 200 mg/kg BW of gallic acid (AG)

R-D (1200) : Diabetic rats treated with 1200 mg/kg BW of EAVa

Effects of the aqueous extract of *Vernonia amygdalina* (EAVa) and gallic acid on the enzymatic activity of catalase in diabetic rats

The results of this study, shown in **figure 4**, show an increase in catalase (CAT) levels in the liver when rats are made diabetic by subcutaneous injection of streptozotocin. Indeed, the catalase levels are 15.23 ± 0.07 UI/mg in healthy rats, before the injection of STZ. After induction of diabetes, the CAT values increased very significantly ($p < 0.001$) in the diabetic rats of the different batches, rising to 26.43 ± 0.08 UI/mg; or 73.54 % increase compared to non-diabetic control rats. These levels remain constant ($p > 0.05$) in non-diabetic or untreated diabetic control rats until the 28th day of experimentation.

The level of catalase in the liver of healthy rats treated with EAVa at 1200 mg/kg BW decreases gradually, but not significantly ($p > 0.05$) until the 14th day of the experiment. From the 21st day of treatment, the decrease in the catalase level becomes significant ($p < 0.05$) with a decrease of 18.25 % (12.45 ± 0.50 UI/mg) compared to that of the control rats healthy, before the treatments (1st day), and this rate is maintained ($p > 0.05$) until the 28th day.

Seven (7) days after the treatment of diabetic rats with gallic acid (AG) at 200 mg/kg BW, the level of catalase decreases to 23.82 ± 0.56 UI/mg ($p < 0.05$), then at 18.3 ± 0.41 UI/mg ($p < 0.01$) on the 21st day; ie a 30.76 % decrease compared to that of the diabetic control rats, then maintained at this value until the 28th day of experimentation. However, on this date, the level of catalase increased by 20.16 % compared to that of the non-diabetic control rats.

When diabetic rats are treated with EAVa at 1200 mg/kg BW, the level of catalase decreases gradually, but not significantly ($p > 0.05$) on the 7th day of experimentation. On the other hand, the catalase level decreases significantly ($p < 0.01$) on the 14th day of treatment (18.63 ± 0.29 UI/mg) and, from the 21st day, the level becomes normal, statistically identical ($p > 0.05$) to that of non-diabetic control rats.

This study thus shows that EAVa, like AG, significantly reduce ($p < 0.01$) the increase in the level of catalase induced by the injection of STZ and, with EAVa, the values return to the normal from the 21st day of treatment. In addition, in healthy rats the catalase level decreased slightly ($p < 0.05$) when given EAVa.

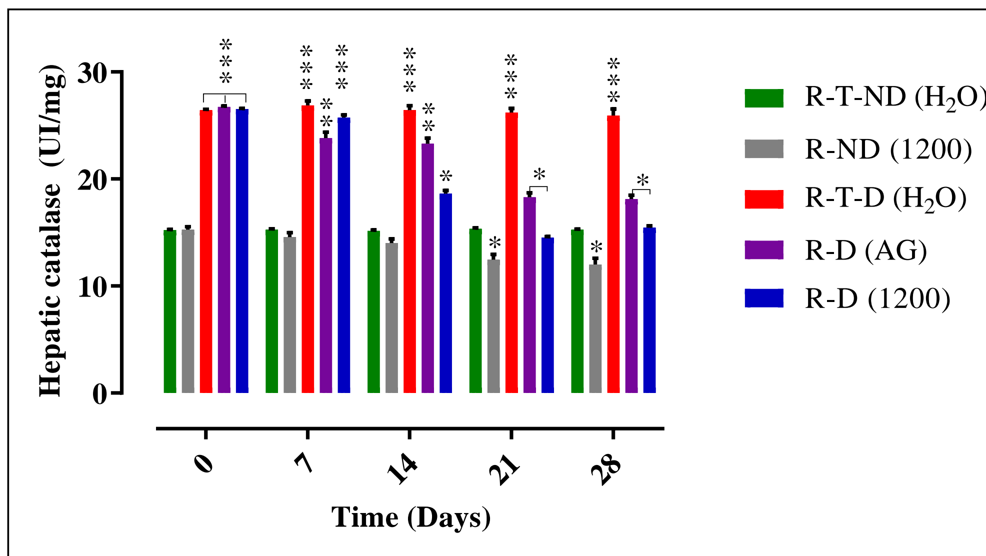

Figure 4: Change in hepatic catalase level in diabetic rats treated with the aqueous extract of *Vernonia amygdalina* (EAVa) or gallic acid

*n = 4; * p <0.05; ** p <0.01; *** p <0.001 compared to non-diabetic controls*

R-T-ND (H_2O): Non-diabetic control rats

R-ND (1200) : Non-diabetic rats treated with 1200 mg/kg BW of EAVa

R-D (H_2O) : Diabetic control rats

R-D (AG) : Diabetic rats treated with 200 mg/kg BW of gallic acid (AG)

R-D (1200) : Diabetic rats treated with 1200 mg/kg BW of EAVa

Effects of the aqueous extract of *Vernonia amygdalina* (EAVa) and gallic acid on the enzymatic activity of superoxide dismutase in diabetic rats

In non-diabetic control rats, the mean value of cytosolic superoxide dismutase (SOD) in rat liver cells is 4.37 ± 0.10 UI/mg. Throughout the experiment (28 days), this rate in these animals varies insignificantly ($p > 0.05$) when they are not treated (**Figure 5**).

In healthy rats, administration of EAVa at 1200 mg/kg BW produced a gradual but small decrease in the level of SOD at the start of the experiment. This decrease becomes significant ($p < 0.05$) from the 21st day of the experiment. Thus, on the 28th day, the level of SOD is 3.67 ± 0.16 UI/mg; ie 16.02 % decrease ($p < 0.05$) compared to that of the non-diabetic control rats before the treatments (1st day).

After the injection of STZ, the level of SOD increased very significantly ($p < 0.001$) in diabetic rats, control and untreated, to approximately 8.30 ± 0.11 UI/mg; or 89.93 % increase over that of non-diabetic control rats, on 1st day. This SOD value was maintained constant ($p > 0.05$) in the diabetic control rats, until the 28th day of the experiment.

In contrast, when diabetic rats are treated with EAVa or gallic acid, SOD levels drop significantly ($p < 0.01$). Thus, with the dose of EAVa at 1200 mg/kg BW or with gallic acid at 200 mg/kg BW, from the 7th day of treatment, the SOD levels respectively drop to 5.47 ± 0.10 UI/mg and 5.61 ± 0.08 UI/ mg; ie respective decreases of 34.10 % and 32.41 % compared to that of the diabetic control rats, then maintained ($p > 0.05$) until the end of the experiment.

This study therefore shows that EAVa caused a small decrease in the level of SOD in healthy rats treated. In addition, when diabetic rats are treated with this extract or with gallic acid, the SOD levels drop significantly but, on the 28th day of treatment, the levels remain higher by 20.98 % and 29.65 % respectively ($p < 0.05$) compared to that of non-diabetic control rats.

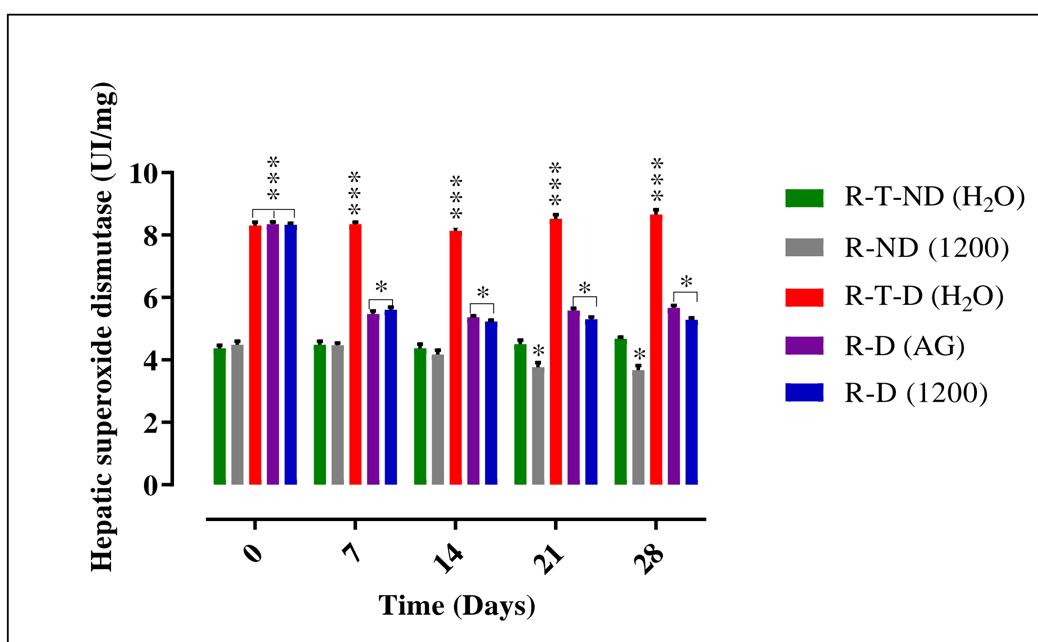

Figure 5: Change in hepatic superoxide dismutase level in rats diabetics treated with aqueous extract of *Vernonia amygdalina* (EAVa) or gallic acid

*n = 4; * p <0.05; *** p <0.001 compared to non-diabetic controls*

R-T-ND (H_2O): Non-diabetic control rats

R-ND (1200) : Non-diabetic rats treated with 1200 mg/kg BW of EAVa

R-D (H_2O) : Diabetic control rats

R-D (AG) : Diabetic rats treated with 200 mg/kg BW of gallic acid (AG)

R-D (1200) : Diabetic rats treated with 1200 mg/kg BW of EAVa

DISCUSSION

An antioxidant is a substance capable of slowing down or inhibiting the oxidation of another substance[8]. The antioxidant power of EAVa, evaluated by assaying the anti-free radical activity by the DPPH test, shows that this extract dose-dependently reduces this free radical. This extract's antioxidant activity is similar to that of quercetin (the standard antioxidant substance). The aqueous extract of the leaves of *Vernonia amygdalina* therefore has an antioxidant power.

The IC_{50} values, obtained from the graph of the percentage of inhibition of DPPH scavenging as a function of sample concentration, indicate that the antioxidant activity of EAVa (IC_{50} = 26.07µg/mL) is greater lower than that of quercetin (IC_{50} = 2.13 µg/mL).

These results are similar to those obtained by authors[9] who, studying the antioxidant activity of extracts from the leaves of *Vernonia amygdalina*, showed that the aqueous extract and the ethyl acetate fraction have the best activity.

The antioxidant activity of EAVa could be due to phytocompounds such as polyphenols, flavonoids and tannins contained in this extract, the antioxidant potential of which has been indicated by several authors. Indeed, according to some authors[10] the antioxidant activity of the aqueous extract of *Vernonia amygdalina* is essentially due to polyphenols. Flavonoids are antioxidants par excellence[11]. These are antioxidants that play an important role in the cell defense system by promoting good stability in osmotic tests on erythrocytes[12]. Tannins promote the absorption of glucose in the peripheral tissue and, through their antioxidant power, they neutralize the effect of free radicals in different tissues to reduce the complications of diabetes[13]. Antioxidant molecules such as ascorbic acid, tocopherol, flavonoids and tannins have also been shown to reduce and discolor DPPH due to their ability to release hydrogen[14]; the intensity of the discoloration determining the potentiality of the trapping of the antioxidants[15].

To determine the antioxidant power *in vivo* of the aqueous extract of *Vernonia amygdalina* (EAVa), the effects of this extract were evaluated in healthy rats and in rats rendered diabetic by injection of streptozotocin (STZ).

STZ, used to induce experimental diabetes, has the ability to induce the excessive formation of reactive oxygen species (ROS) which are highly toxic to cells, especially cell membranes[16]. The injection of streptozotocin causes a reduction in insulin secretion [17]. This insulinopenia causes the degradation of hepatic glutathione (GSH) and therefore a decrease in its activity, an increase in the levels of malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) because insulin acts on the liver by inhibiting glycogenolysis. The significant depletion of the GSH level, following the injection of STZ which triggered the oxidative stress, would probably be due on the

one hand to an increase in its neutralizing action on the free radicals generated and, on the other hand, to decreased synthesis or increased degradation during oxidative stress caused by diabetes[18]. STZ is a generator of free radicals responsible for the oxidation of DNA, lipids and carbohydrates leading to the degeneration of β[19] cells. The increase in oxygen radicals (ROS) could be due either to an increase in their production or to a reduction in their elimination due to the depletion of the antioxidant systems[20]. Other authors[21] report that the activities of SOD and CAT decrease during a state of diabetes mellitus.

Thus, to evaluate the antioxidant power *in vivo*, the effects of EAVa are evaluated on the activity of hepatic glutathione, on the malondialdehyde activity, on the enzymatic activity of catalase and on the enzymatic activity of superoxide dismutase in the liver in normoglycemic rats and in rats rendered diabetic by injection of STZ.

The determination of the oxidative stress parameters shows that in healthy rats, the EAVa at a dose of 1200 mg/kg BW gradually leads, with the continuation of the treatments, to an increase in the level of GSH and a decrease in the levels of MDA, CAT and SOD, compared to those of non-diabetic control rats.

In addition, prior administration of EAVa at a dose of 1200 mg/kg BW to rats rendered diabetic by injection of STZ significantly reduced the fall in hepatic glutathione that was observed in untreated diabetic rats. On the other hand, in the rats made diabetic, but pretreated with EAVa at 1200 mg/kg BW, the levels of malondialdehyde and superoxide dismutase are significantly reduced compared to those of the diabetic control rats, and even this level of SOD almost returns to normal. Also, under the same experimental conditions, the level of catalase normalizes. This improvement in diabetic rats of the parameters of oxidative stress by EAVa confirms the strong antioxidant potential of this extract.

This antioxidant power of EAVa could result from the polyphenols highlighted in this extract. This is in agreement with the work of certain authors[22] who show that rats made diabetic by STZ and treated with polyphenols exhibit high concentrations of GSH. Enzymatic endogenous antioxidants such as glutathione are responsible for detoxifying the body of these deleterious free radicals, and therefore can improve oxidative stress and prevent the development of complications associated with diabetes[23].

In this study, EAVa showed effects similar to those of gallic acid, which is a benchmark antioxidant. Thus, EAVa, like gallic acid, decreases the state of oxidative stress induced by the injection of STZ by repairing oxidative damage by improving the antioxidant system and reducing lipid peroxidation in the liver.

CONCLUSION

The present study was conducted to assess the effects of EVAa *in vitro* and *in vivo* on antioxidant activity in rats. EAVa, like gallic acid, decreases the state of oxidative stress induced, following the injection of STZ, by repairing oxidative damage by improving the antioxidant system and reducing lipid peroxidation at the hepatic level, which allows it to be used against diseases associated with oxidative stress. The antioxidant power of EAVa, demonstrated in this study, justifies the use of *Vernonia amygdalina* (Asteraceae) in the treatment of diabetes and the complications associated with this pathology.

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