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Antidiabetic and Hepatoprotective Effects of an Aqueous Extract of Rauwolfia Vomitoria (Apocynaceae) Roots Barkless on Alloxan-Induced Diabetic Rats

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Abstract | **Original Research Article**

In Côte d'Ivoire, people use Rauwolfia vomitoria (Apocynaceae) as traditional medicine for the treatment of diabetes mellitus. The purpose of this study is to bring out the potentiel antidiabetic effect of an aqueous extract of roots devoid of Rauwolfia vomitoria bark (EARv), in rats made diabetic by alloxan monohydrate, a diabetogen substance, compared with the effect of glibenclamide, a reference antidiabetic substance. For this, the blood glucose level and weight evolution of healthy rats (normoglycemic), diabetic control rats and diabetic rats treated with EARv or glibenclamide are measured, as well as variations in transaminase levels during 28 days of experimentation. Administration of alloxan induces in rats made diabetic characterized by a significant and permanent increase in blood sugar, a decrease in body mass, followed by an increase in the level of serum transaminases, a sign of liver toxicity in rats. In rats made diabetic and treated daily with EARv at a dose of 1000 mg/kg body weight (B.W), there is a decrease in blood sugar and an increase in body mass, in the sense of their normalization, during the 28 days of treatment. In diabetes rats, EARv also results in normalization of serum alanine aminotransferase (ALT) concentration and a significant decrease in aspartate aminotransferase (AST). These effects of EARv at 1000 mg/kg B.W are similar to those of glibenclamide at 10 mg/kg B.W. These results show that, just like glibenclamide, EARv is an antidiabetic, hepatoprotective substance and tends to correct body mass loss in diabetic rats. These effects of EARv justify the use of Rauwolfia vomitoria in traditional medicine in the treatment of diabetes mellitus.

Keywords: Diabetes, antidiabetic, hepatoprotective, Rauwolfia vomitoria.

INTRODUCTION

Diabetes mellitus is one of the main endocrine disorders, characterized by impaired insulin action and deficiency of this hormone (Ajiboyé *et al.*, 2018) and also by dysfunction of carbohydrate, protein and lipid metabolism due to a deficit in insulin secretion (Sada *et al.*, 2013 ; Ajiboyé *et al.*, 2018). These metabolic disorders lead in the long term to pathogenic conditions including micro and macro-vascular complications, neuropathies, retinopathy, nephropathy and a consequent decrease in quality of life and an increase in the mortality rate (Santaguida et *al.*, 2008 ; Airaodion *et al.*, 2018). Diabetes mellitus has long been considered a disease of rich countries. Today this condition affects all social strata of the world's population. According to the WHO, this condition is one of the main killers in the world (WHO, 2013) after high blood pressure and smoking. In 2019, diabetes affected 463 million people worldwide, including 19 million in Africa. If left unable by 2030, there will be more than 29 million Africans with diabetes, and by 2040 more than 47 million, a 143% increase in people with diabetes in Africa (IDF, 2019). These populations continue to treat themselves with medicinal plants. For this reason, WHO recommends that Africans conduct scientific studies to establish a database for plant species used in traditional medicine (WHO, 2013). In Côte d'Ivoire, Rauwolfia vomitoria (Apocynaceae) is a medicinal plant used for the treatment of diabetes mellitus. It is also used to treat

other conditions, including male infertility (Lembe *et al.*, 2014), pancreatic cancer (Yu *et* Chen, 2014).

The objective of this study is to show the effect of Rauwolfia vomitoria aqueous extract on experimentally induced diabetes in Wistar rats and on the liver cells of these animals.

1- MATERIALS AND METHODS

1.1- Equipment

1.1.1- Animal material

The animal material consists of rats Rattus norvegicus (Muridae), of Wistar strain, whose body masses are between 120 and 150 g. They are raised at the pet store of the Laboratory of Animal Physiology, Pharmacology and Pharmacopoeia of the UFR of Natural Sciences of Nangui Abrogoua University (Abidjan, Côte d'Ivoire), at 25 ° C, and under the light day and darkness at night. These rats are fed food provided by the company IVOGRAIN® of Abidjan and have free Access to water. The studies on these rats are conducted in accordance with the European Directive of 24 November 1986 (86/609/EEC) and the Decree of 19 April 1988 (Anonymous, 1986) on the use of experimental animals in research.

1.1.2- Plant material

The plant species used is the root of Rauwolfia vomitoria Afzel. (Apocynaceae) devoid of bark. The root boots of this plant are obtained from the medicinal plant markets in Abidjan. This plant has been identified at the National Center of Floristics (CNF) of the University Félix Houphouët-Boigny (Abidjan, Côte d'Ivoire).

1.1.3- Chemical and pharmacological substances

Glibenclamide (Daonil® 5 mg, Sanofi-Aventis Pharmaceuticals, NJ, USA) and alloxan monohydrate 98% (Sigma Aldrich, France) are used in this study.

1.2- Study methods

1.2.1- Preparation of the aqueous extract of Rauwolfia vomitoria (EARv)

The roots of Rauwolfia vomitoria are stripped of their bark and then dried at room temperature. They are then sprayed using a grinder to obtain a fine powder. Then 200 g of dry root powder devoid of Rauwolfia vomitoria bark is put in 2 L of distilled water. The mixture is stirred for 24 hours at room temperature using a magnetic stirrer. The resulting solution is filtered three times on hydrophilic cotton and Wathman No. 1 filter paper, then dried in the oven (Vacutherm Vacuum Oven, France) at 40 °C. The powder obtained constitutes the aqueous extract of Rauwolfia vomitoria (EARv).

1.1.2- Induction of diabetes by alloxane

Diabetes mellitus is induced in 12-hour fasting rats by a single intraperitoneal injection of alloxane monohydrate, sterile saline, at a dose of 120 mg/kg B.W. Prior to alloxan administration, fasting blood glucose is measured using the Accu-Chek blood glucose meter with test strips. To draw blood, the end of the rat's tail is severed using a pair of scissors. A drop of blood is deposited on the absorbent layer of the test strip, introduced into the glucometer. The value of blood glucose appears on the meter screen. This value is given in g/L. After administration of alloxane, fasting blood glucose is assessed daily, for 1 week, to confirm the diabetic condition of rats. Only rats with blood glucose levels at least 2.50 g/L or more (diabetic rats) are selected for the study of antidiabetic effects of the Rauwolfia vomitoria aqueous extract.

1.1.3- Study of effects of Rauwolfia vomitoria aqueous extract (EARv) on the blood glucose of rats

For this study, 5 healthy rats (non-diabetic) and 15 diabetic rats are used. They divided into 5 batches of rats as follows.

- Batch A: healthy rats receiving distilled water (healthy controls) at a dose of 10 ml/kg B.W. This is the control group.
- Batch B: untreated diabetic rats (diabetic controls). They also receive distilled water at a dose of 10 ml/kg B.W.
- Batch C: diabetic rats treated with 1000 mg/kg B.W. of EARv.
- Batch D: diabetic rats treated with 10 mg/kg B.W. of glibenclamide.

The blood glucose of the animals is measured before the administration of distilled water or tested substances (Day 0) and every 7 days (Day 7, Day 14, Day 21 and Day 28), during 28 days of experimentation. Before each blood glucose measurement, the weight of each rat is recorded.

1.2.4- Determination of serum concentration of alanine transferase and aspartate transferase

The blood of rats used to study antidiabetic effects is also taken from red dry tubes for the determination of biochemical parameters (alanine transferase and aspartate transferase). These blood samples are taken by incision of the tip of the tail of the rats, before the oral administration of the test substances, and every 7 days after the start of treatment, during the 28 days of experimentation. The blood taken from the different tubes is then centrifuged at 6000 revolutions / minute for 15 min and the sera obtained are aliquoted (Gella *et al.*, 1985).

1.2.5- Methods of statistical analysis and plotting of graphs

The data analysis is done using GraphPad Instat software (San Diego CA, USA). The results are given as an average followed by the standard error on the mean (M ± ESM). The difference between two values is determined by the Student-Newman-Keuls test and is considered significant for $p < 0.05$. GraphPad Prism 8 software (San Diego CA, USA) is used to plot graphs.

2- RESULTS

2.1- Effects of alloxan on blood sugar and body mass in normoglycemic rats

Administration of alloxan monohydrate at a dose of 150 mg/kg B.W., induces a permanent and significant increase in blood glucose levels in healthy rats ($p < 0.001$). Indeed, 5 days after treatment, the blood glucose of the rats, which was initially 0.94 ± 0.04 g/L, increases to 3.39 ± 1.70 g/L or a hyperglycemia of 72.27% when rats are made diabetic. This induction of diabetes is accompanied by a non-significant decrease ($p > 0.05$) in the body mass of these rats. Indeed, the body mass of healthy normoglycemic rats is 139 ± 1.3 g. It increases to 133.5 ± 1.4 g 7 days after the injection of alloxan to these animals; a decrease in body mass of 3.40 % when they are diabetic.

2.2- Effects of Rauwolfia vomitoria aqueous extract (EARv) and glibenclamide on blood glucose levels in rats made diabetic

The effects of EARv and glibenclamide on the blood glucose of rats made diabetic by alloxan are shown in Figure 1, compared with the blood glucose of healthy rats and diabetic control rats, during 28 days of experimentation. In healthy untreated rats (healthy controls), which received only distilled water, blood glucose remains constant ($p > 0.05$). It is of the order of 0.98 ± 0.05 g/L throughout the duration of the experimentation (28 days). In untreated diabetic rats (diabetic controls), on Day 0 blood glucose is 3.77 ± 0.44 g/L; an increase in blood glucose of 74.01% ($p < 0.001$) compared to healthy control rats. This blood glucose does not vary significantly ($p > 0.05$) throughout the experiment. It is between 3.66 ± 0.31 g/L and 4.39 ± 0.31 g/L.

In rats treated with EARv at 1000 mg/kg B.W., there is a significant decrease ($p < 0.01$) in hyperglycemia after 7 days of treatment. This drop in hyperglycemia gradually increases over time, to finally give a blood sugar that tends towards normalization. Thus, after 28 days of treatment of diabetic rats with EARv, blood glucose levels from 3.77 ± 0.44 g/L (initial blood glucose, on Day 0), rise to 1.54 ± 0.12 g/L. In rats treated with glibenclamide at 10 mg/kg B.W., it appears, as well as in those who received EARv at a dose of 1000 mg/kg B.W., a gradual reduction in hyperglycemia. Thus, a blood glucose of 1.22 ± 0.01 g/L is measured after 28 days of treatment, against 3.86 ± 0.14 g /L on Day 0. Furthermore, there is no significant difference ($p > 0.05$) between the decrease in induced hyperglycemia in diabetic rats treated with the aqueous extract of Rauwolfia vomitoria and that of diabetic rats treated with the reference antidiabetic substance (Table 1).

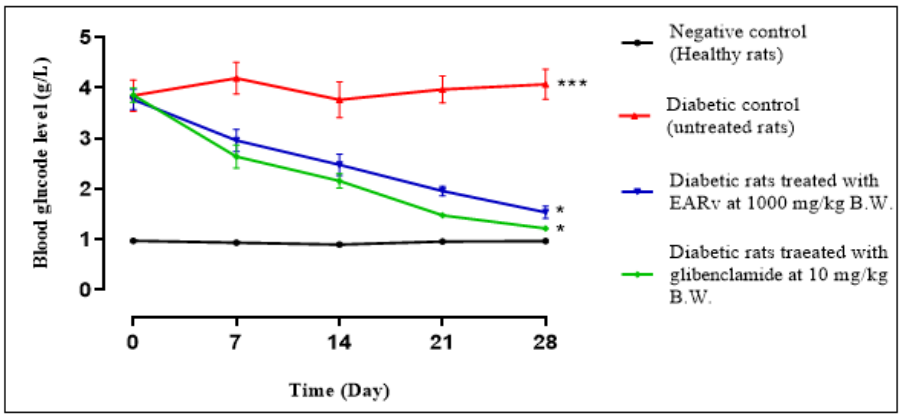

Figure 1: Variation in blood glucose levels in healthy rats and rats with diabetes, whether or not treated with Rauwolfia vomitoria aqueous extract (EARv) or glibenclamide for 28 days

n = 5;*($p < 0.05$);***($p < 0.001$) compared to healthy controls

Table 1: Reduction of hyperglycemia in rats made diabetic for 28 days of treatment with Rauwolfia vomitoria aqueous extract (EARv) or glibenclamide

	Reduction of hyperglycemia treated diabetic rats			
Time / Batches	**Day 0**	**Day 14**	**Day 21**	**Day 28**
Diabetic Rats treated with EARv at 1000 mg/kg B.W.	21,48 %**	51,67 %***	63,13 %***	68,38 %***
Diabetic Rats treated with glibenclamide at 10 mg/kg B.W.	26,46 %**	64,34 %***	70,19 %***	72,70 %***

n = 5;**($p < 0.01$);***($p < 0.001$) relative to Day 0

2.3 Effects of aqueous extract of Rauwolfia vomitoria (EARv) and glibenclamide on the body mass of rats made diabetic

Table 2 shows the change in body mass of healthy rats (non-diabetic rats), diabetic control rats and diabetic rats treated daily with aqueous extract of Rauwolfia vomitoria (EARv) at 1000 mg/kg B.W. or

glibenclamide at 10 mg/kg B.W., during 28 days of experiment.

In healthy control rats, which received only distilled water, after 7 days there was a non-significant increase ($p > 0.05$) in the body mass of animals. After 14 days, the weight gain becomes significant ($p < 0.05$) and, after 28 days, the rate of increase in their body mass is 13.38% ($p < 0.01$) compared to their body mass on Day 0 (139 ± 1.3 g). On the other hand, in rats rendered diabetic untreated (diabetic controls), after 7 days, there is a non-significant decrease ($p > 0.05$) in body mass of 5.29%. Weight loss increases over time and does not become significant ($p < 0.05$) until day 14, with a body mass of 120.4 ± 1.7 g on Day 14, compared to 133.6 ± 1.6 g on Day 0. Thus, on the 28th day, the rate of reduction in the body mass of these animals is 16.24% ($p < 0.01$) compared to their mass on Day 0.

In rats treated daily with EARv at 1000 mg/kg B.W. or glibenclamide (10 mg/kg B.W.), there is a non-significant ($p > 0.05$) and gradual increase in body mass after 7, 14 and 21 days of treatment.

Table 2: Change in body mass of healthy rats and rats with diabetes, treated or not with Rauwolfia vomitoria aqueous extract (EARv) or glibenclamide, for 28 days

	Body mass of rats				
Time / Batches	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Healthy rats (Negative control)	139 ± 1,3 g	45,3 ± 1,2 g	150,2 ± 1,2 g*	153,8 ± 1,2 g*	157,6 ± 2 g**
Diabetic Rats untreated (Diabetic control)	133,6 ± 1,6 g	26,5 ± 2,2 g	120,4 ± 1,7 g*	117,5 ± 1,6 g**	111,9 ± 0,7 g**
Diabetic Rats treated with EARv at 1000 mg/kg B.W.	132,8 ± 1,5 g	33,4 ± 0,9 g	134,6 ± 1,7 g	134,8 ± 0,9 g	146,8 ± 1,3 g**
Diabetic Rats treated with glibenclamide at 10 mg/kg B.W.	134,2 ± 1,1 g	35,2 ±4 g	136,4 ± 2,3 g	138,5 ±1,7 g	148,3 ± 1,3 g**

n = 5;*($p < 0.05$);**($p < 0.01$) relative to body mass on Day 0

2.4- Effects of aqueous extract of Rauwolfia vomitoria (EARv) and glibenclamide on serum alanine aminotransferase concentration in rats made diabetic

The effects of EARv on serum alanine aminotransferase (ALT) concentrations in diabetic rats are shown in Figure 2, compared with levels in healthy rats and diabetic control rats.

Induction of diabetes leads to an increase in serum ALT concentration. Indeed, at the beginning of the experiment (day 0), ALT levels are 100.32 ± 7.83 IU / L and 200.1 ± 10.34 IU / L, respectively in healthy rats and in rats made diabetic; an increase in ALT of 99.46% when rats are made diabetic by alloxan.

Treatment of diabetic rats with EARv at a dose of 1000 mg/kg B.W. results in a non-significant reduction ($p > 0.05$) in ALT up to day 14 of experimentation. This reduction in ALT by EARv becomes significant ($p < 0.05$) from day 21, with a reduction of 37.31%. ($p < 0.001$) on the 28th day. Similarly, the treatment of diabetic rats with glibenclamide (10 mg/kg B.W.) significantly reduces ($p < 0.05$) the ALT level from day 14, and this reduction reaches 40.11% ($p < 0.001$) on the 28th day of the experiment.

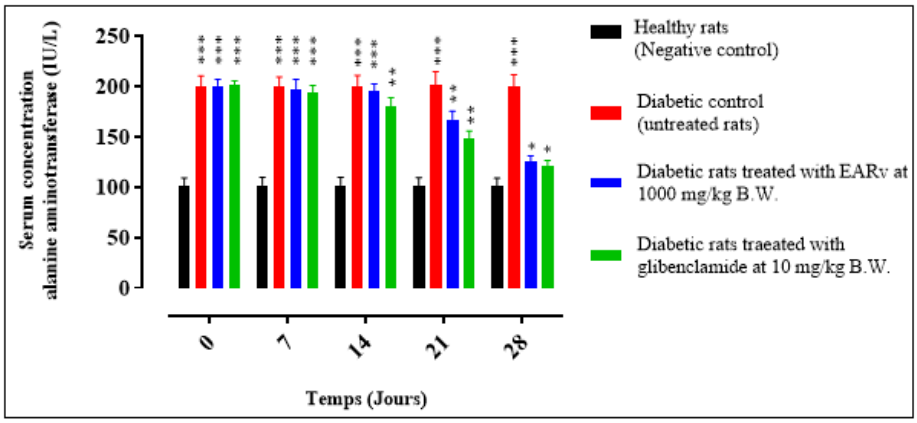

Figure 2: Effects of Rauwolfia vomitoria aqueous extract (EARv) and glibenclamide on serum ALT concentration in healthy rats and rats made diabetic

n = 5;*($p < 0.05$);**($p < 0.01$);***($p < 0.001$) compared to healthy controls

2-5 Effects of aqueous extract of Rauwolfia vomitoria (EARv) and glibenclamide on serum aspartate aminotransferase concentration in diabetic rats

The induction of diabetes by alloxan results in a significant increase ($p < 0.05$) in the serum concentration of aspartate aminotransferase (AST) as shown in Figure 3. On day 0, AST levels are 152 ± 5.34 IU/L and 174.02 ± 4.65 IU/L in healthy rats and rats made diabetic, respectively; an increase in AST levels of 14.48% when rats are made diabetic by alloxane. These AST levels in these rats (healthy and diabetic controls) did not vary significantly ($p > 0.05$) during the 28 days of experimentation. Treatment of diabetic rats with EARv at a dose of 1000 mg/kg B.W. results in a gradual reduction in serum AST concentration during the first 14 days, so that after 14 days of treatment, the AST level returns to normal, identical ($p > 0.05$) to that of healthy control rats, and then remains constant until the end of the experiment. This same result is obtained when diabetic rats are treated for 28 days with glibenclamide 10 mg/kg B.W.

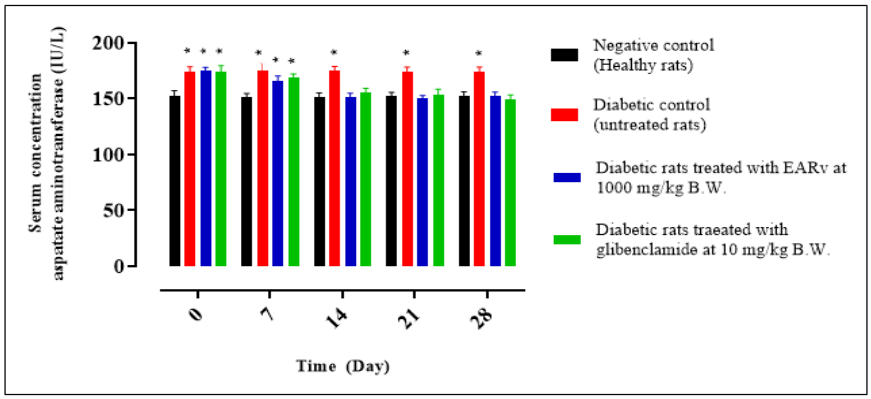

Figure 3: Effects of Rauwolfia vomitoria aqueous extract (EARv) and glibenclamide on serum AST concentration in healthy rats and rats made diabetic

n = 5;*($p < 0.05$);**($p < 0.01$);***($p < 0.001$) compared to healthy controls

3- DISCUSSION

Rats made diabetic by intraperitoneal injection of alloxan monohydrate is an experimental model that allows to study the activity of antidiabetic agents. The action of alloxan is explained by the fact that it induces a decrease in the storage of hepatic glucose by the alteration of insulin secretion l'insuline (Elsner *et al.*, 2000 ; Shetti *et al.*, 2012 ; Kahou *et al.*, 2016). This diabetrogen irreversible induces the destruction of beta cells of the islets of Langerhans located in the pancreas, insulin-producing cells (Grover *et al*., 2000; Salah *et al.*, 2017). The aqueous extract of Rauwolfia vomitoria, administered to rats made diabetic by alloxan, reduces induced diabetic hyperglycemia, and even tends to bring blood sugar back to normal. These results are similar to those of other authors. Chabane *et al*., (2013) showed the antidiabetic potential of the aqueous extract of the aerial part (leaves and branches) of Ajuga iva (Lamiaceae), at a concentration of 0.15 g/ml, in rats made diabetic by alloxan and treated for 3 weeks. Ethanolic extract of Carica papaya (Caricaceae) leaves at doses of 200, 400 and 600 mg/kg B.W. induces a significant reduction in alloxan-induced hyperglycemia in treated rats (Airadion *et al*., 2019). Also, the aqueous extract of the fresh leaves of Pseudarthria hookeri (Fabaceae) at a dose of 1200 mg/kg B.W. leads, after 28 days of treatment, to a significant decrease in blood glucose levels in rats made diabetic (Kahou *et al*., 2016). Airaodion *et al*., (2019) showed the antidiabetic effect of the methanolic extract of the leaves of Telfairia occidentalis (Cucurbitaceae), at doses of 200, 400 and 600 mg/kg B.W., in rats made diabetic with alloxan and treated for 14 days. EARv acts like glibenclamide which is a molecule of the sulfonamide family. Sulphonylancels reduce blood sugar levels in diabetic rats by increasing insulin secretion from the pancreas. Indeed, these molecules bind to a specific receptor, on the membrane of pancreatic β cells in the vicinity of the ATP-dependent potassium duct and cause the closure of the latter. This will lead to membrane depolarization of β cells with opening of voltage-dependent calcium channels and an influx of Ca2+, thus triggering by exocytosis the extrusion of insulin secretion granules (Grimaldi *et al.*, 2009).

During the 28 days of experimentation, diabetic rats treated with the aqueous extract of Rauwolfia vomitoria or glibenclamide showed an increase in their body mass. In contrast, untreated diabetic rats (diabetic controls) lost weight. Weight loss in untreated diabetic rats is associated with diabetes. It is considered a complication of this desease (Momo *et al*., 2006). The aqueous extract of Rauwolfia vomitoria, a potentially antidiabetic substance, is thought to act on diabetic rats, tending to regulate their body mass at the same time as the blood sugar levels of these animals (Garba *et al.*, 2015). These effects are also in favor of the existence of real antidiabetic properties of the aqueous extract of Rauwolfia vomitoria.

Serum assay of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) is very important and well known in the diagnosis and investigation of diabetes. It provides information on the state of functioning of the liver, which plays a major role in the regulation of Blood sugar (Yakubu *et al.,* 2005; Akanji *et al.,* 2013).

Induction of diabetes in rats by alloxane administration leads to an increase in serum alt and AST concentration. This increase is permanent during the 28 days of experimentation. Similar effects of alloxane on ALT and AST levels have also been reported by Asanga *et al*. (2013**)**, Mafulul *et al*. (2013) and Balamurugan *et al*. (2014). The increase in the level of these enzymes in rats made diabetic demonstrates the toxicity of alloxane on the liver, and probable on other organs of these rats. This finding is consistent with several previous (Asanga *et al.,* 2013 ; Mafulul *et al.,* 2013 ; Balamurugan *et al.,* 2014). ALT and AST are transaminases, and are the most commonly used enzyme markers to detect hepatocellular lesions that may be caused by drugs or any harmful compounds in chemicals (Nelson et Lehninger, 2004 ; Khouri et Daradka, 2012). These cytosolic enzymes are widely distributed in tissues with a strong presence in the liver and heart (Eteng *et al.,* 2009). ALT is present mainly in liver cells, making it the main indicator of liver inflammation. AST is present in the cells of the liver, heart and skeletal muscles and other organs. The elevation of their serum concentration is an indication of damage to the liver, and probably to other organs (Foreston *et al.,* 1985 ; Adesokan *et al.,* 2009 ; Adenowo *et al.,* 2014). When their serum concentrations change, the damaged tissues pour these enzymes into the blood plasma.

The aqueous extract of Rauwolfia vomitoria, administered to diabetic rats at a dose of 1000 mg/kg B.W., as well as glibenclamide at 10 mg/kg P.C., gradually decreases serum alt and AST concentrations and, after 14 days of treatment, shows an AST level identical to that of healthy control rats. The sharp reduction in ALT levels in rats treated with EARv and the subsequent normalization of their AST levels indicate that this extract may work by correcting lesions caused by the induction of diabetes by alloxane which is a cytotoxin (Yakubu *et al.,* 2003 ; Mafulul *et al.,* 2013). The aqueous extract of Rauwolfia vomitoria would therefore have a hepatoprotective activity.

Hepatoprotective activities of various plants have been reported by different authors. This is the case for the ethanolic extract of the leaves of Melastoma malabathricum (Melastomataceae) which, at a dose of 300 mg/kg B.W, reduces ALT and AST levels in diabetic rats (Balamurugan *et al.,* 2014). The same is true for the butanolic and methanolic fractions of the ethanolic extract of the leaves of Nauclea latifolia (Rubiaceae), at the respective doses of 100 and 250 mg/kg B.W. (Asanga *et al.,* 2013), and also for the ethanolic extract of the leaves of Gongronema latifolium (Asclepiadeae) at doses of 200 and 400 mg/kg B.W. (Mafulul *et al.,* 2013).

CONCLUSION

The study of the pharmacological effects of the aqueous root extract barkless of Rauvolfia vomitoria on blood glucose shows that this extract has a strong antidiabetic potentiel and is hepatoprotective. This justifies its use in traditional medicine in Côte d'Ivoire and Africa in the treatment of diabetes.

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