

Evaluation Of The Antidiabetic Properties Of The Ethanolic Extract Of The Sclerocarya Birrea Trunk Bark (A. Rich) Hochst And Subchronic Toxicity Of The Kidney And Liver Extract In Wistar Rats

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Abstract— The aim of this study is to evaluate the antidiabetic properties of the ethanolic extract of *Sclerocarya birrea* trunk bark (A. Rich) Hochst and the subchronic toxicity of the extract to liver and kidney in wistar rats. For this, we validated the hypoglycemic and anti-hyperglycemic activity of this extract, as well as its effect on lipid profile, liver and kidney activity with doses of 100, 200 and 300 mg / kg of PC.

Different batches of rats were formed. The raw results are processed by Minitab 1.4 software. The effect of the extract on the parameters is appreciated through the STATA and R software followed by comparison t-tests of two independent samples. The results are expressed as an average $\pm$ Ecartype.

The results show, on the one hand, that the extract has antidiabetic activity at the dose of 100, 200 and 300 mg / kg. After two weeks of treatment, there was a change (significant decrease) in the lipid profile of the rats but with a risk of renal and hepatic involvement, because the activity of the extract increases significantly, the urea creatinine, dASAT, ALT and uric acid.

The ethanolic extract of *Sclerocarya birrea* trunk bark significantly lowered blood glucose levels under physiological conditions or glucose overload conditions.

Keywords—Extract, *Sclerocarya birrea*, glucose, overload, diabetes, Benin

1.INTRODUCTION

For generations, the health care landscape in sub-Saharan Africa has been dominated by poverty and infectious diseases such as malaria and HIV / AIDS. But with changing lifestyles in sprawling urban centers and, increasingly, in rural areas, obesity and diabetes have become a new health priority on the continent. In 1999, the prevalence of diabetes in our country was estimated at 0.1% (Djrolo et al., 1999). In 2012, these figures were multiplied by

46, ie 4.7% for men and 4.5% for women (Djrolo et al., 2012). In addition, WHO estimates that the Africa region, for example, has the highest proportion of undiagnosed diabetes cases, at least 63%. This explains the results of Djrolo, which makes a finding of poor knowledge of diabetes in 75.3% of cases. It must be said that diabetes is booming in the world and Benin this trend is confirmed.

Like cardiovascular diseases, cancer and chronic respiratory diseases, Diabetes is one of the four priority no communicable diseases (NCDs) identified by the World Health Organization (WHO). It is a chronic, incurable, and expensive, constantly rising disease, the complications of which are very debilitating. It is responsible for millions of deaths each year and considerable human misery.

However, it remains a largely preventable disease (FID, 2010).

Today, more than 300 million people around the world suffer from Diabetes. If nothing is done this figure will rise to 500 million in a generation. For example, WHO Resolution AFR / RC50 / R3 of 31 August 2000 (WHO, 2000) encouraged African countries to develop regional strategies on traditional medicine to undertake research on medicinal plants and to promote their uses optimal in health care delivery systems.

We then undertook this study to answer this call. In Benin, many plants have already been used by traditional medicine in the treatment of diabetes. Among these, we can mention the *Sclerocarya birrea*. For the present study which aims to

evaluate the effectiveness of this plant in the treatment of Diabetes, we observed its action on wistar rats whose physiology is the same as that of man. The objective of this work is to evaluate the action of the ethanolic extract of the bark of the *Sclerocarya birrea* trunk on the regulation of blood glucose and to raise the level of subchronic toxicity on the kidneys and the liver in wistar rats.

2. MATERIALS AND METHODS

2.1 MATERIALS

Various materials are involved in the realization of this study, which include, biological material (plant and animal) and laboratory equipment for handling.

Plant Material: It consists of bark of the trunk of *Sclerocarya birrea*. The barks were harvested in August 2017 in the village of Gomez-kparou in N'dali commune in northern Benin by a technical expedition team.

Animal material: consists of the blood (serum), liver and kidneys of Wistar-type rats. These female rats weighing between 150-200g are raised at the Biomembrane Laboratory and Cell Signaling laboratory. All animals are of EOPS sanitary status (free of specific pathogens). Upon receipt, rats are randomly placed in groups of 03 in cages. During this period the animals have free access to food and water and are kept in a pet room at room temperature subjected to a 12 / 12h light / dark cycle.

2.2 METHODS OF STUDY

Obtaining the vegetable drug

The harvested samples were cut into small pieces and then dried at laboratory temperature for three weeks. The barks were powdered and kept in jars to prevent the installation of polluting microorganisms.

➤ Preparation of the extract

To obtain the ethanolic extract of the *Sclerocarya birrea* trunk bark, 100 g of trunk bark powder were weighed using a Sartorius® analytical balance and added to 1 L of ethanol, the whole is brought to a maceration. The macerate is filtered

at the end of each 24h pendant 72h. The deposit is macerated each time until the end of 72 hours. The filtrate obtained is evaporated using ROTAVAPOR at 40 ° C. The recovered extracts are put in an oven at 45 ° C. for drying. After complete drying, the dry extracts attached to the bottom of the dishes are scraped using a stainless steel spatula. The powders obtained are stored in glass vials, sterile and hermetically sealed.



Fig. (1): Ethanolic extract of the bark of the *Sclerocarya birrea* trunk (CHOKKI P. 2018)

➤ Preparation of different feeding solutions

Reference solution

The control solution consists only of distilled water

- Solutions of ethanolic extracts

The solutions of the ethanolic extracts are prepared by introducing 2 g of the crude extract into 20 ml of distilled water. Or at a concentration of 100g / L.

- Glucose solution

The glucose solution is prepared based on D-glucose crystals and distilled water. The concentration of said solution is 571.43 g / l; or 8 g of D-Glucose in 14 ml of distilled water. Subsequently, the mixture is homogenized using a rotary voltex until a completely homogeneous solution is obtained.

- Reference solution (glibenclamide)

The reference solution is prepared by introducing into 5 ml of distilled water a 5 mg glibenclamide tablet.

Determination of the volumes of the different solutions to be administered by experienced dose. Knowing the dose (D) to be tested, the weight (P) of the animal and the concentration (C) of the solution to be administered; the volume (V) of each strain to be administered to a rat is determined from the following formula (Diallo et al., 1989):

$$V = DP/c$$

V: en mL, P: en Kg, D: en mg/kg, C: en mg/mL

Experimentation phase in vivo on the rat wistar

It involved administering the extract to batches of wistar rats at different doses: 100; 200; 300 mg / kg body weight orally. Note that the batches consist of female rats.

The test was performed on two rat models. it's about:

- ☐ normoglycemic rats
- ☐ temporary hyperglycaemia

➤ Test in normoglycemic rats

We realized 4 batches of 3 rats.

Batch 1 (control): Oral administration of distilled water.

Batch 2: Oral administration of the ethanol extract at a dose of 100 mg / kg body weight.

Batch 3: Oral administration of the ethanolic extract at a dose of 200 mg / kg body weight.

Batch 4: Oral administration of the ethanolic extract at a dose of 300 mg / kg body weight.

The various doses were administered each morning (7 days a week) for 14 days. At the end of 14 days, the rats were sacrificed and the target organs (liver, kidneys and heart) were removed and weighed.

Test in rats with Oral Temporary Hyperglycemia (OGTT)

For this study, 15 rats were divided into 5 batches of 3

Orally induced hyperglycemia (OGTT) was performed on all rats used and kept fasting for 16 hours since the previous evening. It consisted in administering by gavage 4g / kg of D-Glucose, diluted in 7 ml of distilled water.

The treatment of the experimental groups by administration of the extracts and of the reference product (Glibenclamide), is done orally, 20min before administering the glucose overload, to make coincide the moment of maximum hypoglycemic activity of the extracts and the product of reference.

Batch I (control): Oral administration of distilled water and 20 min thereafter administration of glucose overload.

Batch II (control): Oral administration of glibenclamide at a dose of 5 mg / kg and 20 minutes after administration of glucose overload.

Batch III: Oral administration of the ethanolic extract at a dose of 100 mg / kg of body weight and 20 minutes after administration of glucose overload.

Batch IV: Oral administration of the ethanolic extract at a dose of 200 mg / kg body weight and minutes after administration of glucose overload.

Batch V: Oral administration of the ethanolic extract at a dose of 300 mg / kg of body weight and 20 minutes after administration of glucose overload.

Glycemia, lipid profile, ASAT and ALAT content of rats were recorded every 30min for 2h.

The blood sample

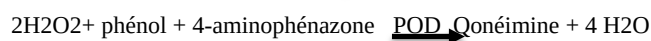
The blood sample is taken according to the experimental protocol used by Weiss et al. (2000), and modified by Descat (2002). Puncture of the retro-orbital sinus was performed and the animal is held in one hand in lateral decubitus, and held by the skin of the neck. The pressure of the thumb on the neck, behind the angle of the jaw, allows compression of the jugular vein, and thus a venous stasis to the head, favoring the filling of the retro-orbital sinus. By lightly pulling on the upper eyelid with the index finger, we create an exophthalmia that facilitates the collection of blood using non-heparinized hematocrit tubes. The end of the tube is slowly introduced into the lateral angle of the eye. The progression through the tissues is facilitated by printing a slight rotation with the pipette. As soon as one reaches the venous plexus, the blood gushes into the periorbital space and rises by capillarity into the tube. The volume of blood collected is 0.5 to 2 ml. Before removal of the tube, the compression is released and the bleeding stops spontaneously when the ocular pressure is normalized. The recovered blood is used for the determination of different biochemical parameters.

Determination of biochemical parameters

➤ Glucose

Glucose is measured after enzymatic oxidation in the presence of glucose oxidase.

The hydrogen peroxide formed reacts with the catalytic action of a peroxidase, with a phenol and 4-aminophenazone to form a red purple quinoneimine compound which serves as a colored indicator, according to the following reactions:



Normal values : 0,70-1,05 g /L

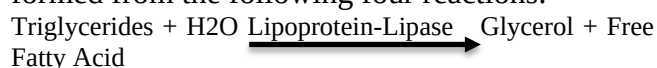
➤ Cholestérol

The cholesterol present in the sample forms a colored complex according to the following reactions:



□ Triglycerides

Triglycerides are determined after enzymatic hydrolysis by lipases. The indicator is a quinone formed from the following four reactions:



+ ADP



Dihydroxyacetone-P + H₂O₂

□ Urea

The technique used is according to the following reactions:



The concentration of plasma urea is proportional to the disappearance of NADH +.

□ Creatinine

The creatinine in the sample reacts with the picrate in an alkaline medium to give a complex in short initial periods, thus avoiding the interference of other compounds.

□ Alanine amino transferase: ALT / GPT

The reagent allows the kinetic determination of alanine transferase activity coupled with a reduced NAD indicator reaction, in 100 mM Tris-HCl buffer pH 7.5, without pyridoxal phosphate, in human serum or plasma. The NADH disappearance rate at 340 nm is measured, which is proportional to the catalytic activity of the GPT.

□ Aspartate amino transferase: ASAT / GOT

The reagent permits the kinetic determination of aspartate amino transferase activity coupled to a reduced NAD indicator reaction in 80 mM Tris-HCl buffer PH = 7.80, without pyridoxal phosphate, in human serum and plasma. The rate of disappearance of NADH at 340 nm is measured, which is proportional to the cytolytic activity of ASAT

3. RESULTS AND DISCUSSIONS

3.1 Extraction efficiency

Tab.(1): Yield of the ethanolic extract of S.birrea trunk bark

Extract	Yield (%)	Couleur	Appearance
Ethanolic	$R = \frac{(22,6 \times 100)}{100}$ R= 22,6%	Dark red	Granular

3.2 Results of phytochemical screening

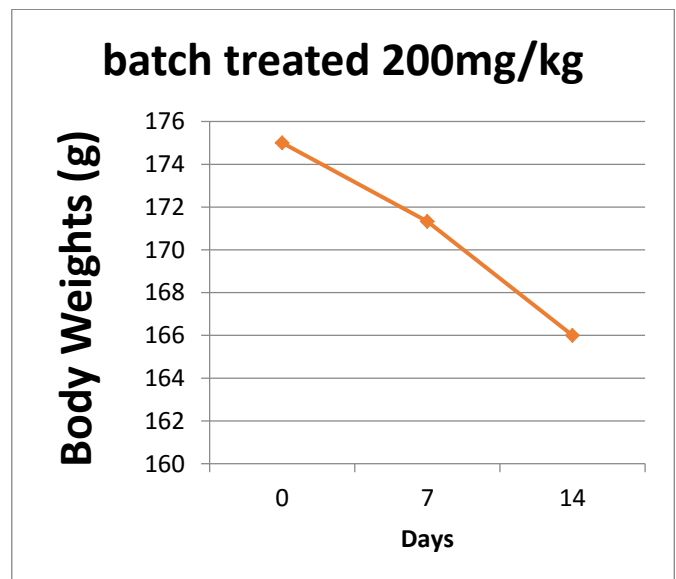
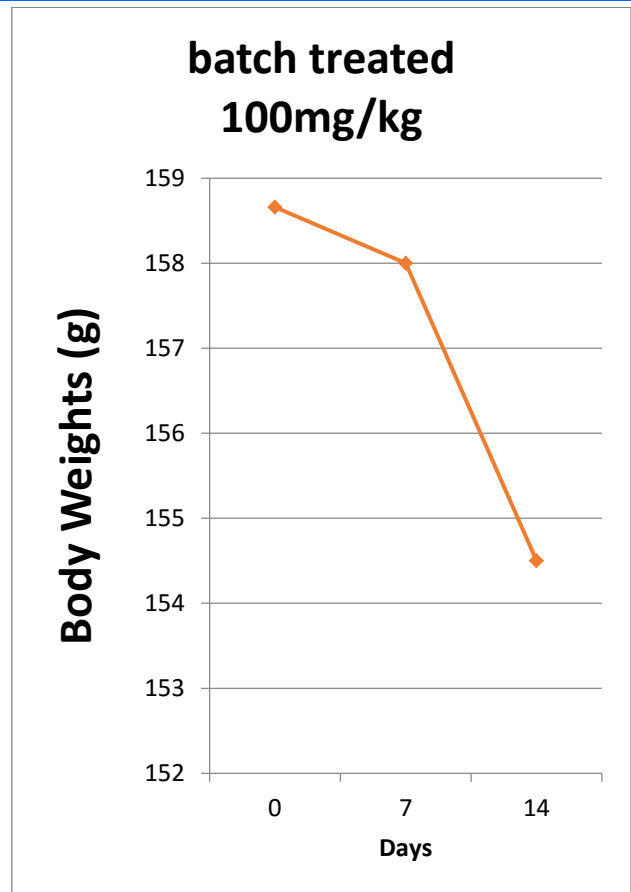
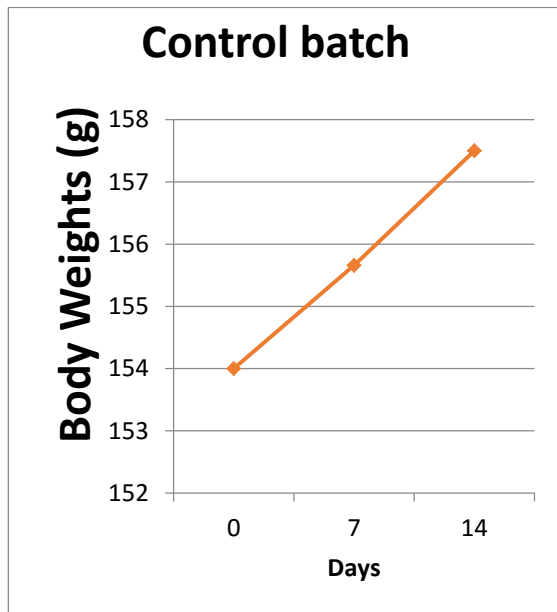
Tab (2): Metabolites identified in the ethanolic extract trunk bark of S. birrea

Secondary metabolites	Observation
Phenolic compounds	+
alkaloids	+
flavonoids	+
anthocyanins	+
Leuco-anthocyanins	+
anthraquinone	+
Reducing compounds gallic	+
	-
	+
Catechism tannins	+
Sterols and terpenes	+
mucilage	-
saponosides	-
coumarins	-

Legend +: Presence of metabolite; -: Absence of metabolite

Phytochemical screening of the ethanolic extract of *Sclerocarya birrea* trunk bark revealed the presence of alkaloids, Catechin tannins, Flavonoids, Leuco-anthocyanins, Sterols and Terpenes.

3.3 Changes in body weight of rats during treatment.



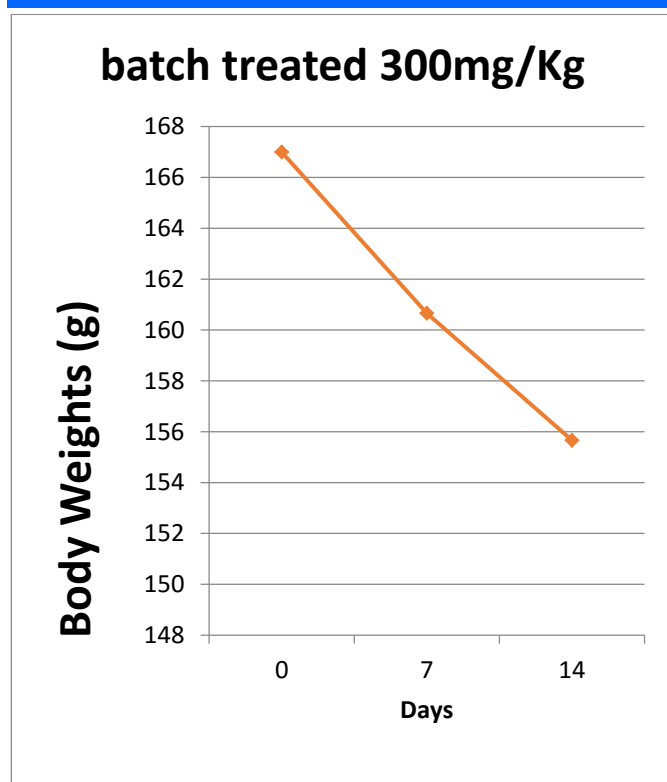


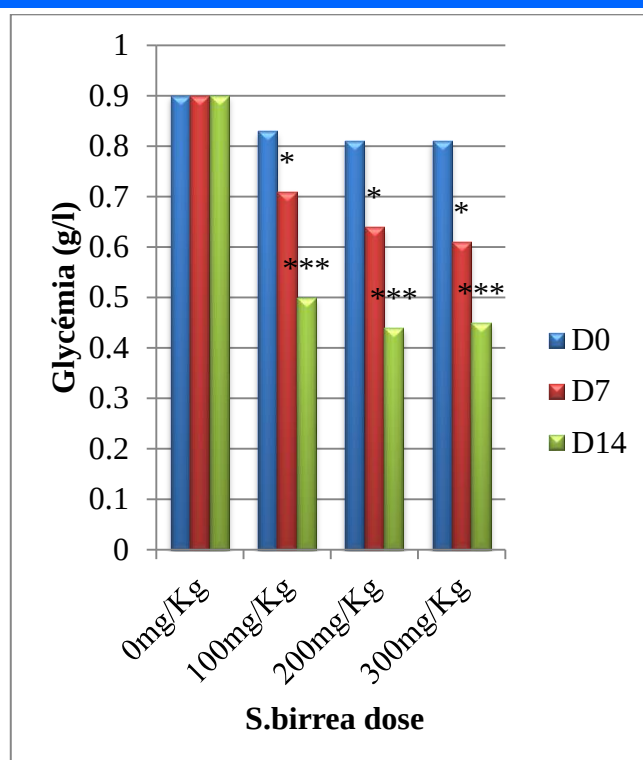
Fig (2): Curves showing the effect of ethanolic extract of *Sclerocarya birrea* on the body weight of rats

We observed an increase of growth in the control rats whereas the weight of the treated rats (100, 200 and 300 mg / kg dc) decreased. The decrease is 2.62% in the rats treated with 100 mg / kg of PC, 5% in those treated at 200 mg / kg of PC and 6.79% in those treated with 300 mg / kg of PC. It should be noted that this decrease is not significant at its doses ($P > 0.05$ in all cases). So it could be said that extracts of *S birrea* slightly reduce or prevent weight gain of rats. This may be due to the lipid-lowering effect (Tomondji et al., 2016) thus inhibiting the accumulation of fat in adipose tissue.

3.4 Determination of antidiabetic activity

-Test in normoglycemic rats

- Blood glucose levels (g / l)

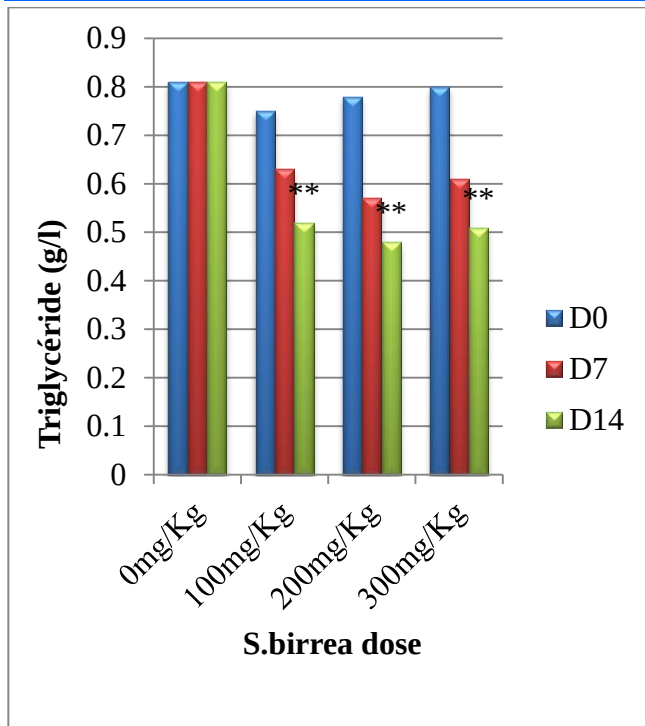


* $p < 0.05$ (significant difference) ** $p < 0.01$ (very significant difference)
 *** $p < 0.001$ (highly significant difference)

Fig(3): Evolution of glycemia in normal control rats and those treated with the ethanolic extract of the bark of the *Sclerocarya birrea* trunk for 14 days.

It is apparent from the analysis of this graph (Figure 3) that the glycemia of the treated rats has decreased. This decrease was significant ($P < 0.05$) at day 7, in rats treated at 100 mg / kg PC and highly significant ($P < 0.001$) in those treated at 200 and 300 mg / kg. At day 14, the decrease was highly significant ($P < 0.001$) at all doses studied (100, 200, 300 mg / kg).

- Triglyceride content (g / l)

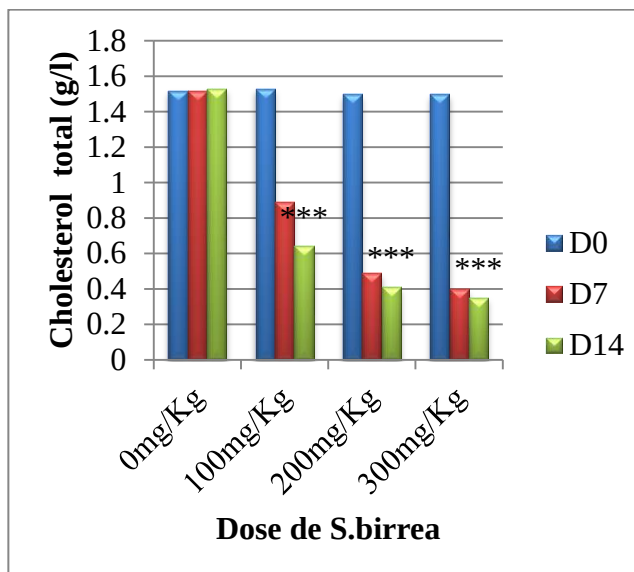


*p<0,05 (significant difference) ** p<0,01(very significant difference) *** p<0,001(highly significant difference)

Fig (4): Evolution of the triglyceride level in normal control rats and those treated with the ethanolic extract of the bark of the Sclerocarya birrea trunk for 14 days.

We can deduct from the interpretation of this figure (4) that the triglyceride level in the treated rats decreases. This decrease is very significant ($P < 0.01$), at all doses tested (100, 200 and 300 mg / kg).

➤ Total cholesterol levels (g / l)

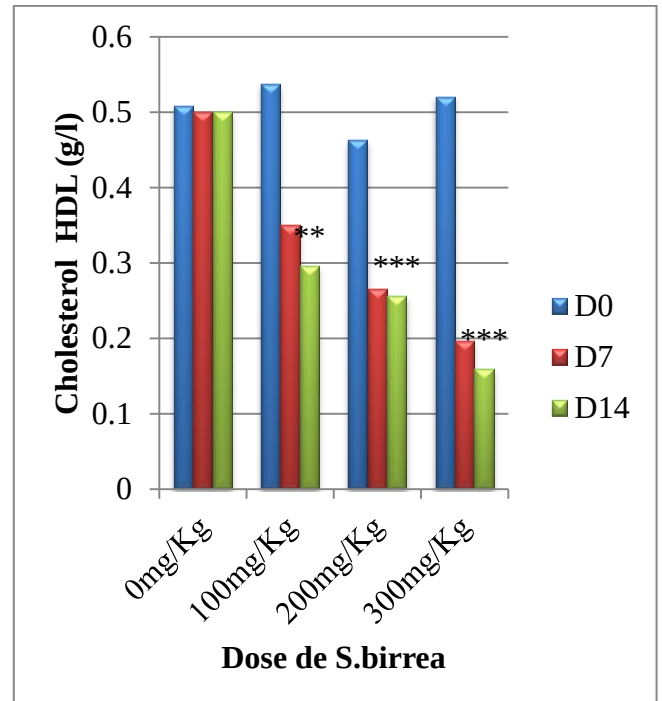


*p<0,05 (significant difference) ** p<0,01(very significant difference) *** p<0,001(highly significant difference)

Fig(5): Evolution of the total cholesterol level in normal control rats and those treated with the ethanolic extract of the bark of the Sclerocarya birrea trunk for 14 days.

In Figure 5 reflecting the evolution of total cholesterol, it can be read that the total cholesterol level decreases. This decrease is highly significant ($P < 0.001$) in all treated rats, regardless of dose.

➤ HDL cholesterol levels (g / l)



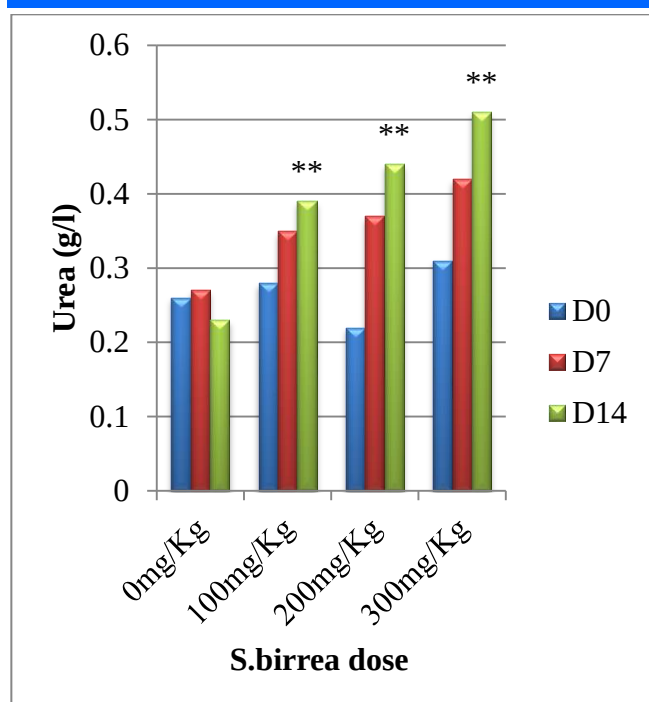
*p<0,05 (significant difference) ** p<0,01(very significant difference) *** p<0,001(highly significant difference)

Fig(6): Evolution of the HDL cholesterol level in normal control rats and those treated with the ethanolic extract of the bark of the Sclerocarya birrea trunk for 14 days.

The analysis in Figure 6 shows that the HDL cholesterol level decreases very significantly ($P < 0.01$) at a dose of 100 and 200 mg / kg. This decrease is highly significant ($P < 0.001$) at a dose of 300 mg / kg.

3.5 Effect of ethanol extract of Sclerocarya birrea on renal and hepatic parameters and target organ weight.

➤ Urea contents (g / l)



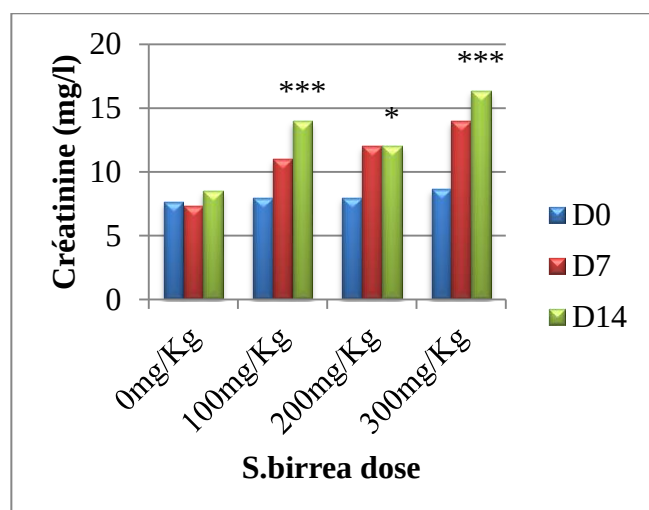
*p<0,05 (significant difference) ** p<0,01(very significant difference) *** p<0,001(highly significant difference)

Fig(7): Evolution of the urea level in the normal control rats and those treated with the ethanolic extract of the bark of the Sclerocarya birrea trunk for 14 days.

From the analysis of figure 7, it appears that whatever the dose, the urea content of the treated rats increases.

No significant difference was noted at day 7 in urea content in rats treated with 100 mg / kg of PC. On the other hand, this increase is very significant ($P < 0.01$) at the dose of 100, 200 and 300 mg / kg after 14 days of treatment.

➤ Creatinine content (mg / l)

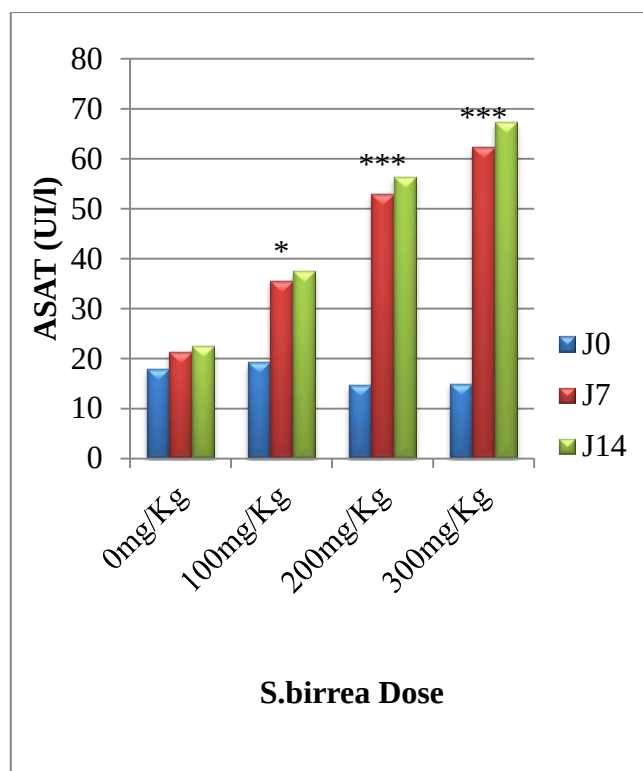


*p<0,05 (significant difference) ** p<0,01(very significant difference) *** p<0,001(highly significant difference)

Fig (8): Evolution of creatinine levels in normal control rats and those treated with ethanolic extract of Sclerocarya birrea trunk bark for 14 days.

According to figure 8, whatever the dose administered, the creatinine level increases. This increase was significant ($P < 0.05$) at the dose of 200 mg / kg and highly significant ($P < 0.01$) at the dose of 100 and 300 mg / kg at 14 days.

➤ ASAT contents (UI / l)

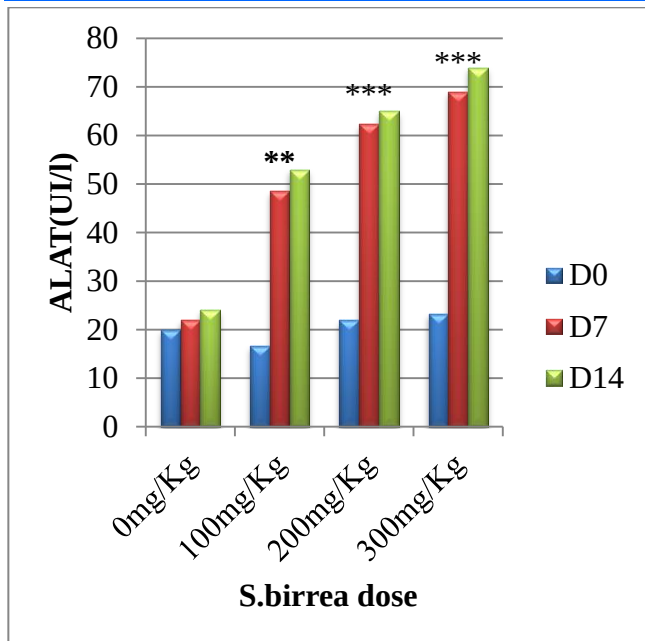


*p<0,05 (significant difference) ** p<0,01(very significant difference) *** p<0,001(highly significant difference)

Fig(9): Evolution of ASAT level in normal control rats and those treated with ethanolic extract of Sclerocarya birrea trunk bark for 14 days.

An increase in ASAT content was observed in all rats. This increase is highly significant ($P < 0.001$) in rats treated at 200 and 300 mg / kg on days 7 and 14.

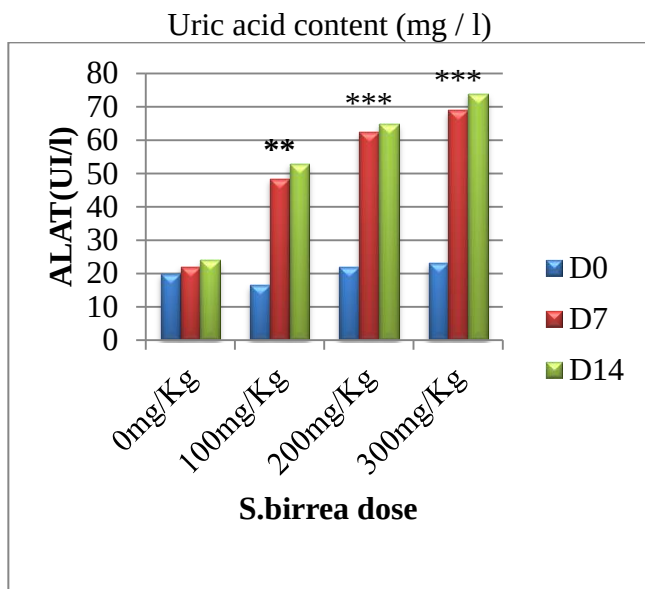
➤ ALAT contents (UI / l)



*p<0,05 (significant difference) ** p<0,01(very significant difference) *** p<0,001(highly significant difference)

Figure (10): Evolution of ALT levels in normal control rats and those treated with ethanolic extract of Sclerocarya birrea trunk bark for 14 days.

There is an increase in ALT content in all rats. This increase was very significant ($P < 0.01$) at 100mg / kg and highly significant ($P < 0.001$) at 200 and 300 mg / kg on days 7 and 14 of treatment.



*p<0,05 (significant difference) ** p<0,01(very significant difference) *** p<0,001(highly significant difference)

Fig(11): Evolution of uric acid levels in normal control rats and those treated with ethanolic extract of Sclerocarya birrea trunk bark for 14 days.

From the analysis of figure 11, it appears that whatever the dose, the uric acid content of the

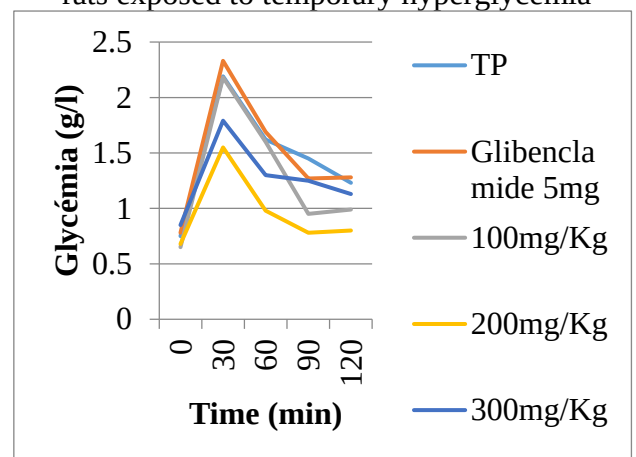
treated rats increases on the 7th and 14th day of treatment. This increase is very significant ($P < 0.01$) at 100 and 200 mg / kg and highly significant ($P < 0.001$) at 300 mg / kg.

Tab(3): Effects of ethanol extract of Sclerocarya birrea on target organ weight

		Relative weight of organs relative to body weight			
		PRF	PRRd	PRRg	PRC
batch	Body weight	Average			
Control	157,5±2,1	0,002	0,002	0,002	0,002
100mg/kg	154,5±0,0	0,02*	0,002	0,002	0,003
			NS	NS	NS
200mg/kg	166±5,56	0,02*	0,003	0,002	0,003
			NS	NS	NS
300mg/kg	155,6±5,8	0,02*	0,002	0,002	0,002
			NS	NS	NS

PRF = relative weight of the liver; PRR = relative weight of the kidneys; PRC = relative weight of the heart; NS = Not significant; Significant with * $P < 0.05$ compared to control group There was a slight increase in liver weight of treated rats compared to control rats.

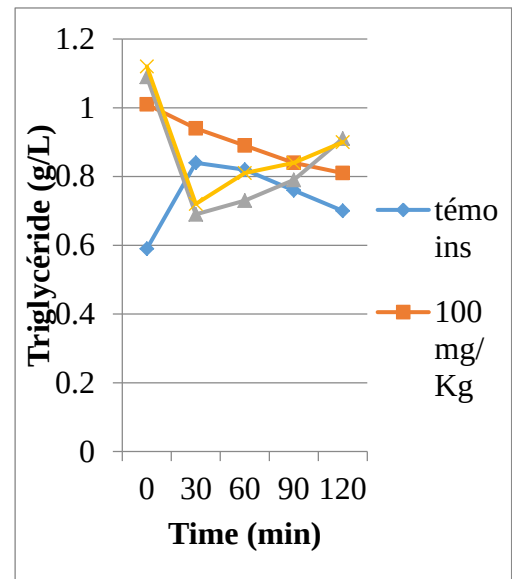
- Oral temporary hyperglycemia test in rats (OGTT)
- Effect of different doses of Sclerocarya birrea on the blood glucose levels of wistar rats exposed to temporary hyperglycemia



Fig(12): Curve showing the effect of different doses of Sclerocarya birrea on blood glucose levels in wistar rats exposed to temporary hyperglycemia.

- For the positive control lot,
-At the beginning of the experiment at $t = 0$ min, before the administration of glucose, the blood glucose of the rats was 0.75 g / l.
- The administration of 4g / kg of glucose leads 30min after a significant increase ($P < 0,05$) of blood glucose up to 2.19 g / l.
- This blood sugar gradually decreases and reaches the values of 1g / l, 2h after the test of HGPVO.
- For reference lot (glibenclamide)
-At the beginning of the experiment at $t = 0$ min, before administration of glucose, the blood glucose of the rats was 7.8 g / l.
-In this batch, the administration of glibenclamide at 5 mg / kg of PC 30 min before the OGTT test induced a hyperglycemia of 2.33 g / l 30 minutes after this test.
- This blood sugar gradually decreases until the values of 1g / l after 2h.
- For the lot having received 100mg / kg of extract
-In this batch, the peak of hyperglycemia obtained in the 30th minute, following the HGPVO test, is 2.18 g / l.
-The blood sugar in this batch is gradually decreasing and reaches the values of 1g / l already 1h30 minutes after the test of HGPVO.
- For the lot having received 200mg / kg of extract.
-In this batch, with the administration of 200 mg / kg of PC, the hyperglycemia peak obtained in the 30th minute, following the HGPVO test, is 1.5 g / L.
- This blood glucose level drops significantly ($P < 0,05$), and reaches the values of 1g / L 1h after the test.
- For the lot having received 300mg / kg of extract
- In this lot, the peak of hyperglycemia obtained at the 30th min, following the HGPVO test, is 1.79g / l.
- The blood sugar level in this batch is gradually decreasing and reaches values close to 1g / l 2h after the HGPVO test.

- Effect of different doses of *Sclerocarya birrea* on triglycerides in wistar rats exposed to temporary hyperglycemia.

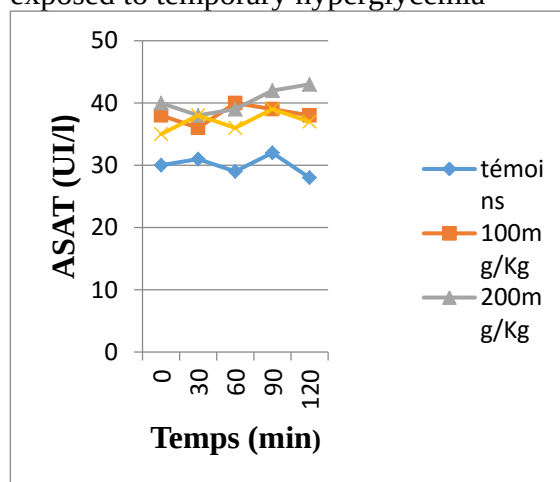


Fig(13): Curve showing the effect of different doses of *Sclerocarya birrea* on the triglycerides of wistar rats subjected to temporary hyperglycemia.

In the control group, the blood triglyceride content, which is 5.91 at the beginning, amounts to 0.82 at $T = 60$ min and then returns to 0.7 at $t = 60$ within batch 3 (100 mg / kg), the triglyceride level is initially 1.01 g / l. It decreases to 0.89 after 1h and then drops to 0.81g / l after 2h. The t-test does not show any significant difference between its values. In batch 4 and 5 the triglyceridemia varies respectively from 1.09 to 0.73 and 1.12 to 0.81 between T_0 and $T + 1h$; then goes up respectively to 0.91 and 0.90 at $T + 2h$. This variation is not significant. The comparison of the mean triglyceridemia between groups and as a function of time shows no difference.

- Effect of different doses of *Sclerocarya birrea* on the ASAT content of wistar rats

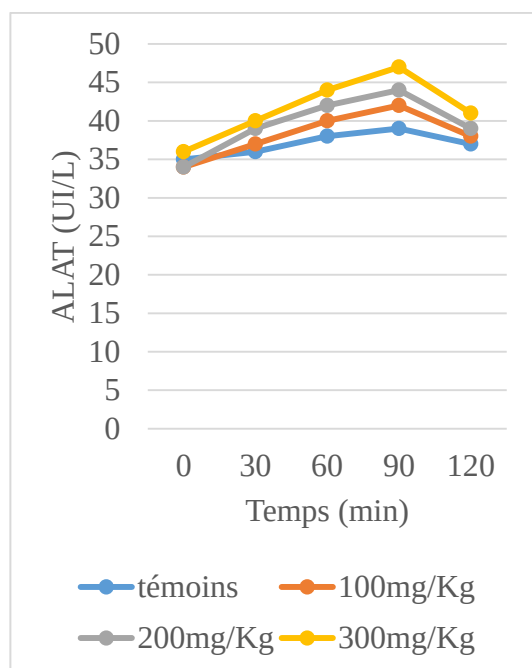
exposed to temporary hyperglycemia



Fig(14): Curve showing the effect of different doses of *Sclerocarya birrea* on ASAT in wistar rats exposed to temporary hyperglycemia.

Figure 14 reveals no significant difference between control and treated batch with respect to the ASAT content.

- Effect of different doses of *Sclerocarya birrea* on the ALAT content of wistar rats exposed to temporary hyperglycemia.



Fig(15): Curve showing the effect of different doses of *Sclerocarya birrea* on ALAT in wistar rats exposed to temporary hyperglycemia.

Figure 30 reveals no significant difference between control and treated batch with respect to ALT content.

3.6 Discussion

Diabetes mellitus is a metabolic disorder whose main feature is hyperglycemia resulting from a lack of secretion, insulin action or both.

Several hundreds of plantes are known and used as anti-diabetics by traditional medicine but a tiny part has been the subject of study or validation of their antidiabetic activities through experiments.

Sclerocarya birrea is a plant that is used for its different effects on blood parameters; in the treatment of several diseases (OJEWOLE et al, 2003).

Results from our study, the ethanolic extract of the bark of the *Sclerocarya birrea* trunk gives a yield of 22.6%. This rate is higher than that of Sanogo et al., (2009) and Sossounon et al (2014) who found respectively 19% and 9.1%. This difference could be due to the nature of the solvents used.

Phytochemical screening reveals that the ethanolic extract of *S. birrea* trunk bark contains the main groups of active ingredients: phenolic compounds, alkaloids, flavonoids, anthocyanins, leuco-Anthocyanins, Antraquinones, compounds reductants, catechin tannins, sterols and terpenes and mucilages. These results are close to those obtained by Gbaglo et al (2016).

The hypoglycaemia observed in normoglycemic rats suggests that ethanolic extracts of *Sclerocarya birrea* have been able to act in the same way as some oral antidiabetic drugs such as glibenclamide by the closure of K^+ / ATP channels, membrane depolarization and influx stimulation. Ca^{2+} , the first key step for the secretion of insulin (Henquin, 2005).

These results are consistent with those of Tomondji et al., 2015, which showed the hypoglycaemic effect of *Sclerocarya birrea* trunk bark on rats subjected to a cafeteria diet. In 2004, Ojewole et al also showed that *Sclerocarya birrea* trunk bark has a dose-dependent hypoglycemic effect in normoglycemic rats and made diabetic by streptozotocin. Sanogo et al., 2008 also showed the effectiveness of decoction in patients with type II diabetes. The antioxidant content

would explain this extra-ordinary effect of our plant.

The hypoglycemic effect of the ethanolic extract of *S. birrea* trunk bark can be assimilated to both the effects of organic constituents and inorganic constituents. Among other things, it is important to note that the inorganic constituents that medicinal plants contain sometimes play a key role in improving their medicinal properties including hypoglycemic activity. Indeed, Bhaskar et al (2008), who have studied the effect of pruriens mucuna << 200 mg / kg >> in streptozotocin-diabetic rats, indicate that a number of essential minerals such as Na, K, Ca, Zn, Mg, Fe, Cu, and Mn may be associated with a mechanism for insulin release and activity. Also, it has been reported that several bioactive molecules isolated from plants such as terpenes and flavonoids influence pancreatic β -cells and stimulate insulin secretion through their antioxidant activities (Bracca et al, 2003, Dagnoko, 2009).

The lipid profile of the rats after a few hours of treatment did not change so much. This shows that the ethanolic extracts of *Sclerocarya birrea* have no instant lipid-lowering activity. In our work, a 14-day treatment of animals causes a significant decrease in lipid profile. These results are consistent with those of Tomondji et al (2015) who have demonstrated a beneficial effect of *Sclerocarya birrea* on the lipid profile of rats. In their work, Tomondji et al (2015) report that many have indicated that an elevation of Total cholesterolemia is strongly associated with coronary atherosclerosis and increases the risk of cardiovascular disease. They enrich their argument by continuing that clinical studies have shown that a decrease in blood cholesterol using a diet or drugs, decreases the incidence of coronary heart disease.

At the doses used, the increase in uremia, uric acid and creatinine may be a renal excretion defect (renal insufficiency). In chronic renal failure, not only is there a deficit in the elimination of urea, but also of sulphates, phosphates and ions (Valdigué, 2008).

The treatment of animals with *Sclerocarya birrea* (ethanol extract) at doses of 100,200,300 mg / kg causes a significant increase in uremia, evidence of kidney damage in these animals. The increase in creatinine is indicative of kidney damage (Valdigué, 2008), supports the idea of a renal

attack as well by the ethanolic extracts of *S. birrea* that the aqueous extracts (Sossounon et al, 2014). These effects seem to be reflected on the mass of the liver. Indeed, in our work, we noted a slight increase in the ratio of liver mass / body mass.

Prolonged treatment at doses greater than 100mg / kg does not seem to affect the kidneys. Some liver function indicators have been explored on *Sclerocarya birrea*. These are alanine amino transferase (ALAT) and Aspartate amino transferase (ASAT). The significant increase in these enzymes (transaminases) reflects the parenchymal involvement by cell necrosis. These results are consistent with those of Sossounon et al (2014) who have proved that the aqueous extract of *Sclerocarya birrea* administered is toxic at doses greater than or equal to 200 mg / kg.

It is concluded that prolonged treatment lasting more than one week may affect vital organs such as the liver and kidneys and would therefore miss its beneficial use.

1. CONCLUSIONS & RECOMMENDATIONS

The search for a solution to health problems has led many researchers to explore the immense richness of flora. In Benin, several plants are used for the treatment of diseases including diabetes. Some of its plants have been scientifically validated for their antidiabetic effect. It generally concerned the verification of their hypoglycemic and anti-hyperglycemic activities.

All of our work has highlighted the hypoglycemic and anti-hyperglycemic activity of ethanolic extracts of the bark of *Sclerocarya birrea* trunk justifying its traditional use as an antidiabetic. It has also allowed us to see the effect of its extracts on the lipid profile, as well as on the kidneys and the liver.

The results show us globally a strong activity on the glycemia and the lipid profile. On the other hand, at the dose of 100, 200 and 300 mg / kg, the characteristic biochemical parameters of the kidneys and the liver are affected. This suggests renal and hepatic impairment for prolonged treatment of *S. birrea* trunk bark, as some authors have observed in other indications.

Our results thus put back on carpet, the question of the insufficiencies of the traditional treatments by the plants and especially the traditional self-medication. These treatments do not take into account the therapeutic specificity of each disease

in relation to the physiology of the vital organs of our organism.

This work will have allowed us to have an idea albeit laminar on the exploration of antidiabetic plants, practical manipulations in biochemistry (clinical) as well as to make a sketch on scientific research.

2. REFERENCES (italicized)

- [1] Abner L.N. Immunologic and genetic factor in type 1 diabetes. *The J. biological chemistry*. 2002, Vol.277.
- [2] ADOMOU AC, SINSIN B, VAN DER MAESEN LJG. Phytosociological and chorological approaches to phytogeography: ameso-scale study in Benin. *Systematics and Geography of Plants*, 2006, 76:155-178.
- [3] Adjanohoun, E.J.; Ahyi, A.; AkéAssi L ; Dan Dicko, L ; Daouda, H ; Delmas, M ; Souzade, S ; Garba, M ; Guinko, S ; Koyong, A ; N'Golo, D ; Raynal, J.L ; Saadou, M. Contribution aux études ethnobotaniques et floristiques au Niger, Médecine traditionnelle et pharmacopée ACCT. 1980, Paris.
- [4] Aharonson, Z., Shani, J et Sulman, F.G. Hypoglycaemic effect on the salt bush (*Atriplex halimus*) a feeding source of the sand rat (*Psammomys obesus*). *Diabetologia*, 1969, 5. PP:379-383
- [5] - Al-Achi, A.. Herbs that effect blood glucose levels. *Women's Health in Primary Care*; 2005 PP:325-330
- [6] Amadou Adiza. Etude d'une recette traditionnelle, des écorces de tronc de *Sclerocarya birrea* Hosch et d'*Uapaca togoensis* Pax utilisées dans le traitement du diabète. Thèse de pharmacie. Bamako, 2007, 101P.
- [7] Arbonnier, M., Arbonnier M : Arbres arbustes et lianes des zones sèches d'Afrique de l'Ouest. France, CIRAD, 2002 .
- [8] Arfa I. Abida Kéfi R., Nouira S. Bali M., Hsouna S., Lasram K., Ben Alaya N., et 2008. Zorgati M. M. Base génétique du diabète ; XI^e Congrès de la société Tunisienne de Médecine Interne.
- [9] Basciano H., Miller A., Baker C., Naples M. and Adeli K. 2009. LXR α activation perturbs hepatic insulin signaling and stimulates production of wapolipoprotein B-containing lipoproteins. *Am. J. Physiol. Gastrintest. Liver physiol.* Vol.297, G, pp323-332.
- [10] Bastard J.P., Maachi M., Lagathu C., Kim M.J., Caron M., Vidal H., Capeau J. et Feve B. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytok. Netw.* 2006 , vol.17, pp4-12.
- [11] Bennett S.T., Lucassen A.M., Gough S.C., Powell E. E., Undlien M. E., Pritchard L. E., Merriman M.J., Kawaguchi y., Dronsfield M.J. et Pociot F. Susceptibility to human type1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat. Genet.* 1995. 2008 vol.9, pp284-292.
- [12] Berhaut, J. Flore illustrée du Sénégal, Vol. 1 (Acanthacée à Avicenniacees). Gouvernement du Sénégal Ministère du Développement Rural Direction des Eaux et Forêts, Dakar. 1971. 626 pp.
- [13] Bhaskar A., Vidhya V. G., Ramya M. . hypoglycemic effect of *Mucun aprurirns* seed extract on normal and streptozotocine-diabetic rats. *Fitoterap.* 2008. Vol.79, pp 539-543.

- [14] Blicklé J. F., Sapin R. et Andrès E. Contribution of total and intact proinsulins to hyperinsulinism in subjects with obesity, impaired glucose tolerance or type 2 diabetes. *Diab. and Metabol.* 2000 Vol. 26, pp 274-280.
- [15] Boden G., Sargard K., Homko C., Mozzoli M., Stein T., P. Effect of a low-Carbohydrate Diet on Appetite, Blood Glucose Levels, and Insulin Resistance in Obese Patients With Type 2 Diabetes. *Ann Intern Med.* 2005. Vol. 142, 6 pp 403-411
- [16] Boitard C. The origin of type 1 diabetes: an autoimmune disease. *Diabetes Metab (Paris).* 2002 .Vol. 414, pp 813-820.
- [17] Brownlee M. Biochemistry and molecular cellular biology of diabetic complications. *Nat.* 2001. Vol. 414, pp 813-820.
- [18] Coulibaly, B. 1988. Contribution à l'étude des remèdes traditionnels utilisés dans le traitement du diabète au Mali, Thèse de pharmacie, Bamako (Mali), N°88, 113 p
- [19] Cox N. J., Wapelhorst B., Morrison V.A., John L., Pinchuk L., Spielman R.S., Todd J.A. et Concanon P. Seven regions of the genome show evidence of linkage to type 1 diabetes in a consensus analysis of 767 multiplex families. *Am. J. Hum. Genet.* 69:820-830. 2001.
- [20] Cuny, P., Sanogo, S., Sommer, N., Arbres du domaine soudanien. Leurs usages et leur multiplication, CRRAS, Sikasso (Mali), 1997. 122 p.
- [21] Dagogo-Jack S. et Santiago J.V. Physiopathology of type 2 diabetes and modes of action of therapeutic interventions. *Arch intern med.* 1997. Vol. 157, pp 1802-1817.
- [22] Dagnoko, S. Etude de la qualité des feuilles de *Sclerocarya birrea* utilisées dans le traitement du Diabète. Thèse Pharmacie, FMPOS, Université de Bamako, 2009 Mali. 118p.
- [23] Dao, A. Etudes botaniques et phytochimiques de *Sclerocarya birrea* (A. Rich). *Hochst (Anacardiaceae).* 1998. Thèse de pharmacie
- [24] Dey Lucey, M.D., Anaja, S., Attele, D.D.S et Chun-SuYuan, M.D. (2002). Alternative therapy for type 2 diabetes. *Alternative Medicine Review*; 7(1). PP:45-48.
- [25] Dimo T, Rakotonirina SV, Tan PV, Azay J, Dongo E, Kamtchouing P, Cros G.. Effect of *Sclerocarya birrea* (Anacardiaceae) stem bark methylene chloride/methanol extract on streptozotocin-diabetic rats. *J Ethnopharmacol.* 2007. 110(3):434-438
- [26] Donath M., Gross D., Cerasi E. et Kaiser N. Hyperglycemia-induced beta-cell apoptosis in pancreatic islets of *Psammomys Obesus* during development of diabetes. *Diab.* 1999. Vol. 48, pp 738-744.
- [27] Donath M.Y. Les diabètes de type 1 et 2: parallèles moléculaires, catalogue Flammarion Médecine-sciences, 2004. Editions Flammarion 246p.
- [28] Eloff, J.N., Antibacterial activity of *Marula Sclerocarya birrea* (A. Rich.) Hochst. Subsp. *caffra* (Sond.) (Anacardiaceae) bark and leaves. 2001. *Journal of Ethnopharmacology* 76(3): 305-308.
- [29] Fomba Mama. Contribution à l'étude de l'activité hypoglycémisante des feuilles d'une plante antidiabétique (*Sclerocarya birrea*) (A. Rich). *Hochst. Anacardiaceae.*

2001. Thèse de pharmacie. Bamako. 63p.
- [30] Galvez Peralta, J.; Zarzuelo, A.; Busson, R.; Cobbaert, C.; De Witte, P. (-)-Epicatechin-3-galloyl ester: a secretagogue compound from the bark of *Sclerocarya birrea*. *Planta Medica*. 1992 . 58(2), 174-175.
- [31] Gondwe M, Kamadyaapa DR, Tufts M, Chuturgoon AA, Musabayane CT. *Sclerocarya birrea* [(A. Rich.) Hochst.] [Anacardiaceae] stem-bark ethanolic extract (SBE) modulates blood glucose, glomerular filtration rate (GFR) and mean arterial blood pressure (MAP) of STZ-induced diabetic rats. *Phytomedicine*. 2008. 15(9):699-709.
- [32] Gouwakinnou et al. Local knowledge, pattern and diversity of use of *Sclerocarya birrea*, 2011. *Journal of Ethnobiology and Ethnomedicine*, <http://www.ethnobiomed.com/content/7/1/8>
- [33] Gueye, M. *Contribution à l'étude pharmacodynamique d'une plante antidiabétique Sclerocarya birrea* (A. Rich). Hochst., 1973. Thèse doctorat sciences pharmaceutiques. Dakar. Sénégal.
- [34] **Grimaldi André. Philippe Cornet. Nathalie Masseboeuf. Marc Popelier. Claude**
- [35] **Guillausseau P-J. Maes T., Virally M., Laloi- Michelin M., et Kevorkian J. Ph.(2007).** Physiopathologie des complications du diabète. *Louvain Méd.* Vol.126(3), S, pp 34-36.
- [36] **Hagopian W. A., Michelsen B., Karlsen A.E., Larsen F., Moddy A., Grubin C.E.,(1993).** Auto antibodies in IDDM primarily recognize the 65,000-M(r) isoform of glutamic acid than the 67,000M(R) isoform of glutamic acid decarboxylase. *Diab.* Vol.42, pp631-636.
- [37] **Fr.m.wikipedia.org**
- [38] **Hall JB, (2002).** *Sclerocarya birrea* (A. Rich) Hochst. In *Record from Protabase*. Edited by: Open LPA, Lemmens RHMJ. Wageningen, PROTA.
- [39] **Hamadi N.(2010).** Effet du resveratrol sur les décentes antioxydantes chez les rats rendus diabétiques par l'injection de la streptozotocine, Thèse de Doctorat de l'Université Mentouri Constantine, pp3-4.
- [40] **Henquin J. C., (2005).** Le traitement pharmacologique du diabète de type2 : mode d'action des médicaments d'aujourd'hui et de demain, *Louvain Méd.*, vol.124, S, pp39-46.
- [41] Henquin J.C., Ravier M.A., Nenquin M., Jonas J. C., Gilon P. Hierarchy of the β -cell signals controlling insulin secretion. *Eur J Clin Invest*. 2003. Vol.33, pp 742-750.
- [42] Hotamisligil G. S., Shargil N. S., Spiegelman B. M., Adipose expression of tumor necrosis factor alpha: direct role in obesity linked insulin resistance. 1993. *Scien.* Vol. 259pp87-91.
- [43] Ihara Y., Toyokuni S., Uchida k., Odaka H., Tanaka T., Ikeda H., Hiai H., Seino Y., Yamada Y., Hyperglycemia causes oxidative stress in pancreatic beta-cells of GK rats, a model of type 2 diabetes. 1999 *Diab.* vol.48, pp 927-932.
- [44] Ito T. Recent advances in the study of the fine structure of hepatic sinusoidal wall: A review. 1973. *Gunma Rep Med Sci*. Vol6,pp 119-163.

- [45] Iwu, Handbook of African Medicinal plants, 1993. Edition CRC Press, London. 401p
- [46] Jackus V., et Rietbrock N. Advanced Glycation End-Products and the Progress of Diabetic Vascular Complications. *Physiol. 2004. Res.* Vol. 53, pp 131-142.
- [47] JOHN AO, OJEWOLE cardiovascular journal of South Africa. 2006. Vol 17, N°3. 117-123.
- [48] Kahn B.B. Facilitative glucose transporters: regulatory mechanisms and dysregulation in diabetes. 1992. *JClin Invest.* Vol.89, 1367-1371.
- [49] Kathleen G. Type 1 diabets: pathogenesis and prevention. 2006. *CMAJ.* Vol.175, pp 165-170.
- [50] Keita Aminata. Etude de trois plantes utilisées dans le traitement traditionnel de l'ulcère gastroduodénal dans le district de Bamako : *Borassus oethiopum* Mart (Palmeae), *Sclerocarya birrea* (A. Rich). *Hochst. (Anacardiaceae)* et *Ximenia americana* L. (Olacaceae), 2005 Thèse de pharmacie, Bamako, 173P.
- [51] KERHARO J, ADAMS G. (1974) La pharmacopée Sénégalaise traditionnelle. Plantes médicinales et toxiques. Edition Vigot et frères. Paris 1011 p.
- [52] KRINKE GJ. the Laboratory Rat (Handbook of Experimental Animals), Academic Press, « History, Strains and Models » 2000, 3–16 p.
- [53] Lam D. H., Watkins E.R., Hayward p., Bright J., Kerr N., Parr-Davis G., Sham P.A. A randomized controlled trial of cognitive therapy of relapse prevention for bipolar disorder: outcome of the first year. *Archives General Psychiatry*, 2003, vol, 60, pp145-152.
- [54] Laurens, A. Sur des Anacardiacees africaines et malgaches, *Poupartia birrea*, *Pourpartia caffra* et *Anacardium occidentale* (Etude particulière des polyphénols des feuilles), 1976. Thèse doctorat, Pharm. (Etat), Paris.
- [55] Maedler K., Oberholzer J., et Bucher P. Monounsaturated Fatty Acids Prevent the Deleterious Effects of Palmitate and High Glucose on Human Pancreatic beta-cell Turnover and Function. 2003. *Diab.* Vol.52, pp 726-733.
- [56] Marles.R. Jet Farnwork.N.R..Plants as sources antidiabetic agents. 1994. Ed con Med Plant Res; PP:179-187.
- [57] Maedler K., Spinas G. A., et Lehmann R.Glucose induces beta-cell apoptosis via up regulation of the Fas-Receptor in human islets. 2001. *Diab.* Vol. 50, pp 1683-1690.
- [58] Mandrup-Poulsen, T..Recent advances : Diabetes. *Biol and Med J.* Vol.316, pp 1221-1225. 2003
- [59] Matschinsky F.M.et Daniel P.jr. Glucokinase activators (GKAs) promise a new pharmacotherapy for diabetics; *Med. Reports.* 2010. Vol.2, pp 43-48.
- [60] Michel R. Le diabète de type 1. *Médecine Nucléaire.* 2001. Vol 25, pp 95-101.
- [61] Nepom G. et Kwok W.. Molecular basis for HLA-DQ association with IDDDM. *Diab* vol.47, pp1177-1184. 1998
- [62] Nienke V.D.W., Frans G.M., Kroese J. R., Jan-Luuk H. Viral infections as potential triggers of type 1 diabetes. *Diabetes/Metabolism Res and Revi.* Vol.23, pp169-183. 2006.

- [63] Ogbobe, Okoro. Physico-chemical composition and characterization of the seed and seed oil of *Sclerocarya birrea* Plant Foods Hum. Nutr. 42(3), 201-6. 1992.
- [64] Ojewole J. A.O. Evaluation of the anti-inflammatory properties of *Sclerocarya birrea* (A. Rich). Hochst. (family: Anacardiaceae) stem-bark extracts in rats in journal of Ethnopharmacology, (85) 217-220 p. 2002
- [65] Ojewole JA. Hypoglycemic effect of *Sclerocarya birrea* [(A. Rich.) Hochst.] [Anacardiaceae] stem-bark aqueous extract in rats. 10(8):675-681. 2003
- [66] Ojewole JA. Evaluation of the analgesic, anti-inflammatory and anti-diabetic properties of *Sclerocarya birrea* (A. Rich.) Hochst. Stem-bark aqueous extract in mice and rats. 2004.
- [67] Ojewole JA. Vasorelaxant and hypotensive effects of *Sclerocarya birrea* (A. Rich.) Hochst (Anacardiaceae) stem bark aqueous extract in rats. Cardiovasc J S Afr. 17(3):117-123. 2006
- [68] Paquot N., et Tappy L. Adipocytolines: Link between obesity, type 2 diabetes and atherosclerosis. Rev Med Liege. Vol. 60, pp 369-373. 2005
- [69] Parkan, J. Dendrologie forestière 2ème partie, cours destiné aux élèves Ingénieurs des sciences appliquées, Edition PNUD/UNESCO-MLI-65/504, Katibougou, tome II, 255p. 1974.
- [70] Peter-Riesch B., Philippe J., Stalder H. Découverte d'un diabète sucré. Prim. Care. Vol.2, pp 284-290. 2002.
- [71] Pincemail J., Meurisse M., Limet R., et Defraigne J. Evaluation du stress oxydant : une réalité pour le médecin généraliste. Vaiss., Cœur, Poum. Vol.4, pp 148-154. 1999.
- [72] Pugliese A., Eisenbarth G.S. Type 1 diabetes mellitus of man: genetic susceptibility and resistance. Adv Exp Med Biol. Vol.552, pp 170-203. 2004.
- [73] Recep O., Sera S., Zerrin O., Fahri K., Meltem C. The influence of type 1 diabetes mellitus on dentition and oral health in children and adolescents. Yonsei Med J. Vol.49, pp 357-365. 2008.
- [74] Roep B.O., Duinkerken G., Schreuder G.M., Kolb H., De Vries R. R., et Martin S. HLA-associated inverse correlation between T cell and antibody responsiveness to islet autoantigen in recent-onset insulin-dependent diabetes mellitus. Eur J Immunol. Vol.26, pp 1285-1299. 2007.
- [75] Sachon. Guide pratique du diabète. Editions médicales spécialisées. Directeur éditions et multimédia : José Vieira. 376p. 1998
- [76] Samba Sanogo. Etude de la phytochimie et de l'effet hypoglycémiant de trois (3) plantes utilisées dans la pharmacopée traditionnelle au Mali, 2008.
- [77] Sanogo, S .Etude de la phytochimie et de l'effet hypoglycémiant de trois plantes utilisées dans la pharmacopée traditionnelle au Mali, Thèse Pharmacie, FMPOS, Université de Bamako, Mali. 118p. 2007.
- [78] Smith GC, Clegg MS, Keen CL, Grivetti LE Mineral. Values of selected plant foods common to southern Burkina Faso and to Niamey, Niger, West Africa. Int J Food SciNutr. 47(1):41-53. 1996.
- [79] She J.X., et Marron M.P. Genetic susceptibility factors in type 1 diabetes: linkage disequilibrium and functional analyses. Curr Opin Immunol. Vol.10, pp 682-699. 1998
- [80] Thompson, K. Het Godin, D.V.. Micronutrients and antioxidants in the

- progression of diabetes. *Nutrition Research*,15(9).PP :1377-1410. 1995
- [81] Wautier J.L., Guillausseau P.J. Advanced glycation end-products and their receptors and diabetic angiopathy. *DiabMetab.* Vol.27, pp 535-539. 2001
- [82] Wautier J.L., Zoukourian C., Chappey O., Wautier M.P., Guillausseau P.J., Cao R., Hori O., Stern D., Schmidt A.M. Receptor mediated endothelial cell dysfunction in diabetic vasculopathy: soluble receptor for advanced glycation end-products blocks hypermeability.*JClin Invest.* Vol.96, pp 238-243. 1996.
- [83] Wautier M.P., Massin P., Guillausseau P.J., Huijberts M., Levy B. I., Boulanger E., Laloi-Michelin M., et Wautier J.L.N (carboxymethyl) lysine as a biomarker for microvascular complications in type 2 diabetic patients. *Diabetes Metab.* Vol.29, pp 44-51. 2003
- [84] Yansambou, Hamsatou, Etude des constituants des feuilles de *Ziziphus mauritiana* Lam. (Rhamnaceae) utilisées traditionnellement dans le traitement du diabète au Mali, thèse de pharmacie, Bamako, 2002, P.82.
- [85] Yaro. B. Contribution à l'étude du traitement traditionnel du diabète au Mali. Thèse de pharmacie. Bamako133p. 1992.