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Impact of the aqueous extract of the bark of Sclerocarya birrea on the lipid membrane structure hepatocytes Wistar rats

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ABSTRACT: The purpose of this study was to evaluate the impact of aqueous extracts of the stem bark of Sclerocarya birrea on the lipid membrane structure of the hepatocytes of Wistar rats. For this, we made the aqueous extraction with the powder of the dried bark of Sclerocarya birrea by the method of Bintou M. (2010). We proceeded to the serum transaminase testing for biochemical examinations then we realized the isolation of rat hepatocytes by cold trypsinization method and lipid extraction drawing inspiration from the method known as solvent gradient used by Stephan et al. (2004) for extracting mycobacterial lipids that has been adapted to the hepatocytes. And finally the impact of aqueous extracts of the barks of Sclerocarya birrea on the lipid membrane structure hepatocytes Wistar rats was assessed by thin layer chromatography of cellular lipids after oral administration of aqueous extracts from barks of Sclerocarya birrea rats Wistar to 50, 100, 200, 300 and 400 mg / Kg.

The results of our work have shown that aqueous extracts from the bark of Sclerocarya birrea rise to any increase in serum transaminase levels that would be due to necrosis of hepatocytes. This is confirmed by the study of the lipid profile of the membranes of rat hepatocytes using the technique of thin layer chromatography of lipids revealed that high doses ⩾200mg / kg aqueous extract of the stem bark of Sclerocarya birrea would cause damage in hepatocytes. The aqueous extract of trunk bark Sclerocarya birrea would be toxic and the toxicity of the extract depend on the administered dose; because at 50mg / kg and 100 mg / kg lipid profile did not change significantly compared to the negative control. So the aqueous extract administered Sclerocarya birrea be toxic at doses greater than or equal to 200mg / Kg.

KEYWORDS: Sclerocarya birrea, bark, membran lipid, Benin

I. INTRODUCTION

Africa is known for its rich flora, diverse and abundant resource of valuable plants used for therapeutic purposes by the indigenous population. In fact, of the 300,000 plant species found on the planet, over 200,000 species live in tropical African countries and have proven medicinal properties (Sofowora 1993). One can not talk about health in Africa without the use of medicinal plants. Medicinal plants are a valuable heritage for humanity and especially for the majority of poor communities in developing countries who depend on it for their primary health care and subsistence (Salhiet al., 2010). As weak economic resources of the people in these countries limit the purchase of pharmaceuticals (Diallo, 2014) However, the main problem with conventional treatments, including herbal, is the lack of knowledge with respect to efficiency, the mode of action, the active ingredients, the dosages to use, indications, safety and quality control. Nearly 80% of the natural substances used in the biomedical field come from tropical plants (Campa, 2005). These observations require African states the need to redefine health policy remained too long and based exclusively on the import of pharmaceutical products in recomposing much as possible with ancestral medicinal practices of their land. Medicinal plants identified with people and boasting good therapeutic renowned should therefore be put to the test of serious investigations of chemical and biological decryption that will provide evidence of their safety and that will make their much more efficient uses.

Sclerocarya birrea is a native medicinal plant from Africa whose virtues were already the subject of several studies. And studies have shown antidiabetic properties Sclerocarya birrea (Coulibaly, 1988; Haidara, 1999; Fomba, 2001), the glucose lowering activity of aqueous extract of the stem bark of the plant in rats (Ojewole 2003 ; Ojewole, 2004); anti diarrheal activities of the extracts of the stem bark of the plant (Galves et al., 1991); secretogogue the activity of the ester (-) - epicatechin isolated -3-galloyl the trunk bark of the plant (Galvez et al., 1992).

These potentials revealed the Sclerocarya birrea stimulate our interest in the search for a possible relationship between some active ingredients of the plant and the functioning of the liver, the liver being a

regulatory body, the main detoxifying organ of the body and a affect its operation affects the overall health of an individual. This justifies the purpose of this work: "Impact of the aqueous extract of the bark of Sclerocarya birrea on the lipid membrane structure of the hepatocytes of Wistar rats."

II. MATERIALS AND METHODS

Plant Material

This is Sclerocarya trunk bark of birrea, harvested in July 2015 at the village of Gomez-kparou in the town of N'dali northeast Benin by a technical team in expédition.Les bark after receipt were washed and subjected to an oven at 40 ° C for 72h for drying. These dried peel were finely crushed and the powder obtained is then stored in glass jars to prevent the installation of microorganisms pollutants.

Animal material

These liver Wistar type rats subjected to a sub-chronic gavage (14 days) and high at the pet of Biomembranes Laboratory and Cell Signaling FAST / UAC. Breeding is done in a lighted room 12 hours a day, and the temperature is ambient. The animals had free access to food and water.

Methods

Préparation of the aqueous extract of the bark of troncde Sclerocarya birrea
To obtain the aqueous extract of the bark of the trunk Sclerocarya birrea, leaf powder 100g were weighed with an analytical balance Sartorius® and added to 1L of water, is brought to the boil for 30 minutes. The decoction is cooled for 20 minutes, filtered through gauze and cotton on. The resulting filtrate was overthrown in dishes and then put in the oven for evaporation at 50 ° C. After complete drying, the solids joined in the bottom plates are scraped with a spatula in stainless steel, then kept in bottles already labeled glass. The powders obtained were stored in glass vials, sterile and hermetically sealed.
For each series of experiments the extract is weighed and dissolved in distilled water to give solutions in the desired concentrations.

Gavage rats

In this handling, it is to administer the aqueous extracts of the barks of Sclerocarya birrea to Wistar rats (female) of about 200g of the same diet, and orally at various doses for about two weeks. These animals received no other medication in the time outside the extract.
- The rats were randomly divided into 06 lots, 03 rats (# 1, # 2 and # 3), then the doses per kg body weight per day are attributed to lots respectively, 0 mg / Kg; 50 mg / kg; 100 mg / kg; 200 mg / kg; 300 mg / kg; 400 mg / kg; and the volume is fixed to be administered at V = 01 ml.
- Then we weigh each rat of each batch to find the average weight and calculate the effective dose of extract to be administered to him. Then gave daily and at the same time the rats for 14 days.
- Lot 1 is the control group and receives oral administration of distilled water instead of the aqueous extract (0 mg / Kg) of Sclerocarya birrea for 14 days;
- Other lots 2; 3; 4; 5 and 6 are treated by oral administration at doses of 50 mg / kg; 100 mg / kg; 200 mg / kg; 300 mg / kg; 400 mg / kg body weight per day of the aqueous Sclerocarya birrea extract for 14 days

Figure 15: Picture of feeding a rat (Photo Amos Oluwagbemiga S., 2015).

- Finally, the rats were sacrificed 24 hours after the last feeding, to take our livers for various manipulations.

Isolation of rat hepatocytes by cold trypsinization technique [25; 26; 27; 28; 29]

The trypsinization is the method for disaggregating the tissue to isolate cells using the hot-exposure method or cold. Due to tryptic activity of trypsin in the hot method of trypsinization at 37 ° C; the tissue is damaged and harvesting the cells after 30 minutes incubation may lead to a reaggregation or damage cells. These inconveniences can be overcome by employing a minimum concentration of trypsin and a long incubation time to increase the efficiency of separation of trypsin. In a longer incubation time, the tryptic activity affects the cells and therefore the temperature should be minimized to reduce the effect triptych. The easy way to reduce damage to the cells during exposure is to soak the tissue with trypsin at 4 ° C for 6-18 hours to allow penetration of the enzyme with little triptych activity followed by exposure of 20 to 30 minutes at 37 ° C for disintegration.
Rats (approximately 200 g) were anesthetized by inhalation of chloroform, and the liver was isolated by a horizontal incision on the ventral surface near the diaphragm of the animal.

The liver of dead time does not last more than 2 to 3 min. The liver was kept in saline phosphate buffer solution (PBS) at 4 ° C until use. The experimental environment was sterilized.
0.375 g liver was removed and then cut with the surgical blade into small pieces; these pieces were washed with PBS and then transferred to other Petri dishes and unwanted tissue such as fat tissue and necrotic tissue were removed. liver pieces were washed once more with PBS; transferred to a falcon tube in which they are soaked in 0.25% crude trypsin in RPMI for 18 hours at 4 ° C. Trypsin is added following the ration of 10 ml per 1 g of tissue.

After incubation, the liver pieces were exposed at 37 ° C for 30 minutes in 1 ml of warm RPMI per 100 mg of tissue. In order to completely disperse the tissue, a gentle pipetting is performed, then the solution is filtered through a sterile muslin cloth to remove the larger pieces. Cells were preserved for experiments and stored in common crop in 5% dimethyl sulfoxide (DMSO) used as cryoprotectant, added to the cell to reduce the formation of ice and thereby prevent cell death in during the freezing process, and the cells were frozen at -80 ° C.

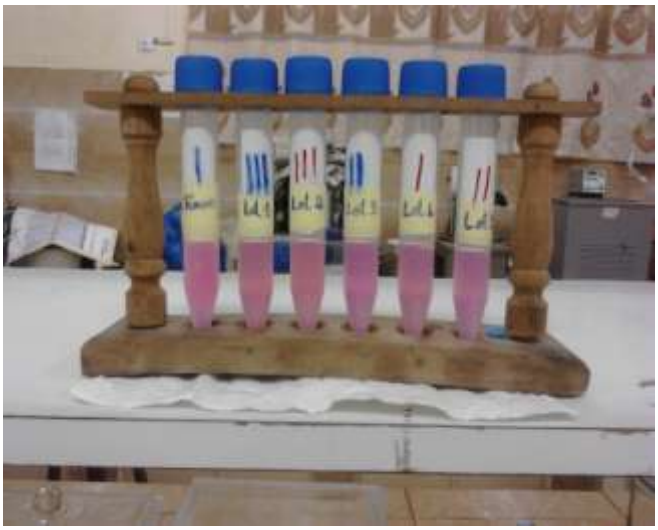


Figure 16: liver cells of rats Photo isolated and stored in culture in 5% DMSO (Photo Amos Oluwagbemiga S., 2015).

Extraction and thin layer chromatography of cellular lipids Lipid extraction

The lipid extraction method is based on the method called solvent gradient used by Stephan et al. (2004) for extracting mycobacterial lipids. It has been adapted for hepatocytes. Four successive extractions are carried out on the mêmeéchantillon cells. The extraction solvent is a methanol / chloroform increasingly enriched in chloroform [11; 29].
- Cellular lipids were extracted by four successive extractions of a day, stirring at 250 rpm. The lipid extract is recovered after separation of the cells; adding an aqueous solution of NaCl 0.9% (volume proportional to CHCl 3) afforded the two phases [30; 31] and the cells are then ready to undergo the next extraction.
- The proportion of solvents chloroform (CHCl3) and methanol (CH3OH) are different depending on the extracting step:

o First extraction: CHCl 3 / CH 3 OH (1V: 2V)
o Second extraction: CHCl 3 / CH 3 OH (1V: 1V)
o Third extraction: CHCl3 / CH3OH (2V: 1V)
o Fourth extraction: CHCl3 / CH3OH (2V: 1V)
A volume V = 15 ml for 500 mg of cells
- All the organic phases were mixed and the solvent is evaporated in a Rotavapor and under hood.
- The lipid extract is resuspended in a volume of chloroform (1 ml solvent per 1 g of lipids).
- Part of the lipid extract is used for analysis and the other is stored at -80 ° C.
The sample was stored at - 80 ° C is evaporated under a stream of nitrogen and then resuspended in chloroform to know the precise volume before use analysis of lipids [11].

Figure 17: Phase separation photos (below) containing chloroform lipids by settling.

Figure 18: Photograph of the lipids extracted from solutions in solvent (chloroform) evaporates.

Thin layer chromatography (TLC) of cellular lipids This is the separation of different classes of silica gel lipids by thin layer chromatography. CCM is based on electrostatic interaction / hydrogen bonding. The principle of "like attracts like that," often encountered in chemistry still helps explain here the nature of the phenomena involved. It is based on the differential adsorption phenomena: a mobile phase consisting of solvent progresses along a stationary phase (silica) fixed on a plate. TLC takes place in three stages: preparation of the tank, preparation of the plate, and elution. The different cellular lipids are divided into two main groups: phospholipids and neutral lipids. They are separated on two different plates. [11]

Separation of neutral lipids by TLC

As their name suggests, the neutral lipids are not charged compounds. They include: the free or esterified fatty acids, mono-, di- and triglycerides and free and esterified sterols. They are separated using low-polar solvents: hexane, ethyl ether, ... The separation of neutral lipids is done first by a migration in a hexane solvent: ethyl ether: glacial acetic acid (40: 10: 1 v / v / v) up to half laplaque. The plate is dried and then is

performed a second migration up to 1 cm from the upper edge of the plate with hexane alone [11; 31].
• Preparation of the tub (mobile phases)
- Eluent E1: it corresponds to the mixture M1, the following solvents: hexane: ether: ethyl: glacial acetic acid (40: 10: 1 v / v / v) [11; 31].
- Eluent E2: it is the only hexane. [11]

The two eluents are placed in two different tanks C1 and C2 at a height of about 0.5 cm from the bottom of the vessel; and the tanks are closed so that they are saturated with vapor eluant for 10 to 15 min.
• Preparation of the stationary phase

Silica plate (Silica gel 60, Merck, France) of dimensions 10 × 10 cm, is drawn in pencil, a line about 1 cm from the bottom edge and parallel thereto; then placed on the line, using capillary tubes and 0.5 to 1 cm apart, 10 mL of each lipid sample (control, sample 1, sample 2 ...) [11; 31].
• Elution or development of the chromatogram
- The plate is placed slightly oblique position in the C1 tank is then closed. The solvent which covers the bottom rises along the plate by capillary action.
- Migration is stopped when the solvent front reaches about half of the plate.
- The plate is then placed in the second tank C2 after drying and migration is stopped when the solvent front reaches about 1cm of the upper end of the plate.
- The level reached by the solvent (front line) is marked by a thin line and the plate is dried in the open air or using a drier [11; 31].

Figure 19: Photographs showing a first eluting in hexane: ethyl ether: glacial acetic acid (40: 10: 1 v / v / v) up to half of the plate (left) and a second elution in hexane until a cm from the upper edge of the plate (right).

2.3.4.2.1. Separation of phospholipids by TLC

Phospholipids are polar molecules separated using highly polar solvent combinations: water, methanol, chloroform ... The migration was carried out at once with a mixture: Chloroform / methanol / ammonia (32.5: 12.5: 2). Such proportions are at the limit of the two-phase mixture. We must remain in monophasic [11; 31].
• Preparation of the vessel (mobile phase)

The eluent is the mixture of the following solvents: chloroform / methanol / ammonia (32.5: 12.5: 2). The eluent was placed in a C3 tank at a height of about 0.5 cm from the bottom of the vessel; then closes the tank so that it is saturated with steam eluent during 10 to 15 min [11; 31].

• Preparation of the stationary phase

Silica plate (Silica gel 60, Merck, France) of dimensions 10 × 10 cm, is drawn in pencil, a line about 1 cm from the bottom edge and parallel thereto; then placed on the line, using a capillary tube and 0.5 to 1 cm apart, 10 mL of each lipid sample (control, sample 1, sample 2, ...)

• Elution or development of the chromatogram

- The plate is placed slightly oblique position in the C3 tank is then closed. The solvent which covers the bottom rises along the plate by capillary action.
- Migration is stopped when the solvent front reached approximately 1 cm in the upper end of the plate.
- The level reached by the solvent (front line) is marked by a thin line and the plate is dried in the open air or using a drier [11; 31].

Figure 20: Photograph showing elution in chloroform / methanol / ammonia (32.5: 12.5: 2) to one cm from the top of the plate.

Revelation of lipids on TLC plates

The detection is carried out by spraying a copper sulfate solution II 10% (10 g CuSO4 5H2O in 100 mL of H3PO4 8% (v / v)). Once the dry plate, it is placed for about 15 min in an oven at 150 ° C min. The presence of spots indicates the presence of lipids in question. [11

III. RESULTATS

The extraction of Return is calculated using the formula: R = *Mass* of *l'extract* / *Mass* of powder of bark × 100

Extract	Yied,%	Color	Appearance
Decoction / water	**R= 27,3×100/300 R= 9,1**	**Red wine**	**Powder**

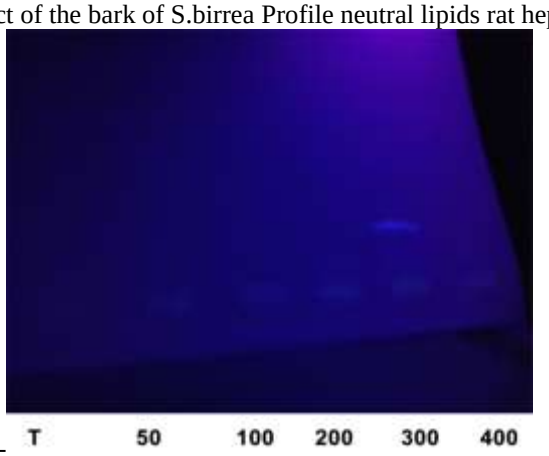
The yield of 9.1% obtained for extraction is low

Mass lipids extracted

Essay	Témoin (0mg/ Kg)	Lot 1 (50mg /Kg)	Lot 2 (100mg / Kg)	Lot 3 (200mg / Kg)	Lot 4 (300mg / Kg)	Lot 5 (400mg / Kg)
AverageMass of lipid extracts	**0.132 mg**	**0.196 mg**	**0.237 mg**	**0.131 mg**	**0.084 mg**	**0.152 mg**

Table showing the average masses of lipids extracted from Rat Hepatocytes

3.3. Action of the aqueous extract of the bark of S.birrea Profile neutral lipids rat hepatocytes



II-

Figure 21: Photograph of the plate to UV 366 nm before revelation with CuSO4 II, showing the migration of different neutral lipids. (Photo of Oluwagbemiga Amos S., 2015)

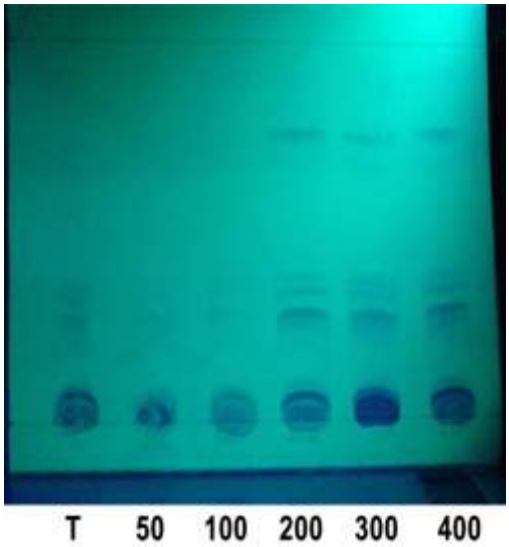


Figure 22: Picture of the plate with UV 254 nm before revelation with CuSO4 II, showing the migration of different neutral lipids. (Photo of Oluwagbemiga Amos S., 2015)

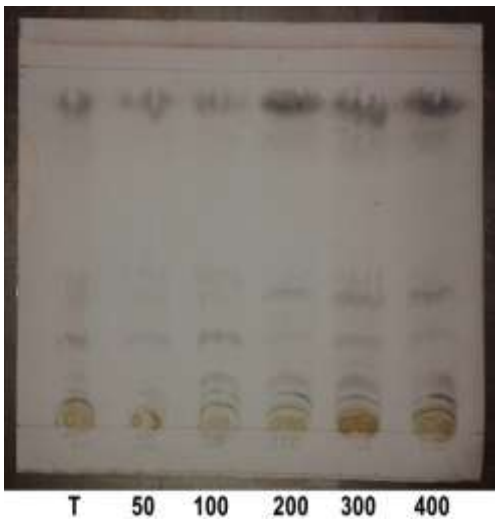


Figure 23: Photo of the plate after development with CuSO4 II, showing the migration of different neutral lipids. (Photo of Oluwagbemiga Amos S., 2015)

Rats batches treated with the extract, it was found that the daily administration of different doses of ethyl extract from the bark of Sclerocarya birrea relative to the control group during 14 days influenced qualitatively and quantitatively different types of lipids neutral in cells. The figure 23; 24 and 25 show the pictures of the plate obtained UV 260 nm respectively, UV 340 nm and after revelation with CuSO4 II after a first migration with hexane / diethyl ether / glacial acetic acid (40: 10: 1 v / v / v) up to half of the plate, then a second migration with hexane alone to 1 cm from the edge of the plate. This allows to separate the different neutral lipids. The first deposit corresponds to the negative control and the other are respectively the doses of 50mg / kg, 100mg / kg, 200mg / kg, 300mg / kg and 400mg / Kg of ethyl extract from the bark of S. birrea. Whereas in the neutral lipids in their entirety is found that:

- To witness so there's about five spots five neutral lipids
- For doses of 50mg / kg and 100mg / kg was also about five spots so five neutral lipids
- For doses of 200mg / kg, 300mg / kg and 400mg / kg was about seven spots so seven neutral lipids

Considering each lipid, note that:

- The spots at the witness are very low, so each lipid in question is small amount compared to other higher doses.
- The spots at doses of 50mg / kg and 100mg / kg are as low as those of the control relative to the other, so the amount of each of the lipids in these doses is almost the same as in the control over witness
- The spots at doses of 200mg / kg, 300mg / kg and 400mg / kg are sharper, darker compared to the control and the first deus doses so the amount of each lipid at these doses is greater by compared to the control and all other doses.

In addition, we note that at each deposit there are more heavy lipids (in low spots) as light lipids (on high spots).

IV. ACTION ETHYL EXTRACT OF THE BARK OF SCLEROCARYA BIRREA PROFILE PHOSPHOLIPIDS RAT HEPATOCYTES

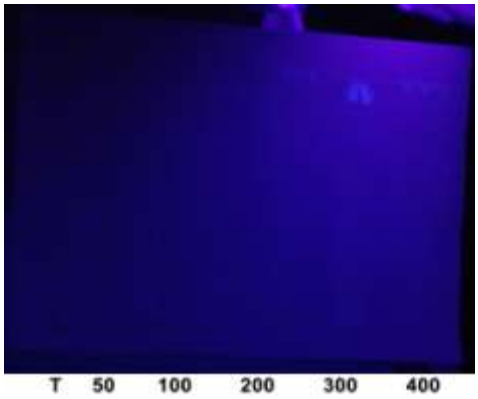


Figure 24: Picture of the picture plate of the plate to UV 366 nm before revelation with CuSO4 II, showing the migration of various phospholipids. (Photo of Oluwagbemiga Amos S., 2015)

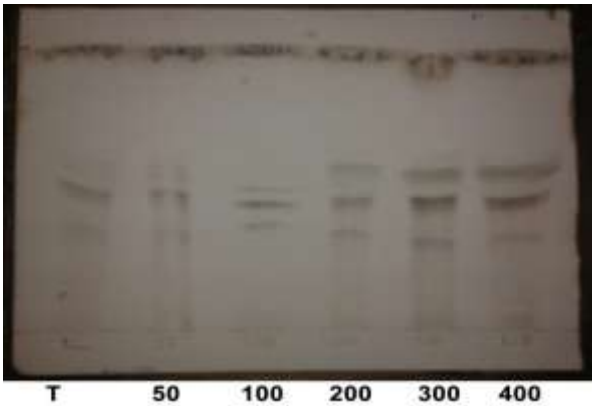


Figure 25: Photograph of the picture plate of the plate with UV 254 nm before revelation with CuSO4 II, showing the migration of various phospholipids. (Photo of Oluwagbemiga Amos S., 2015)

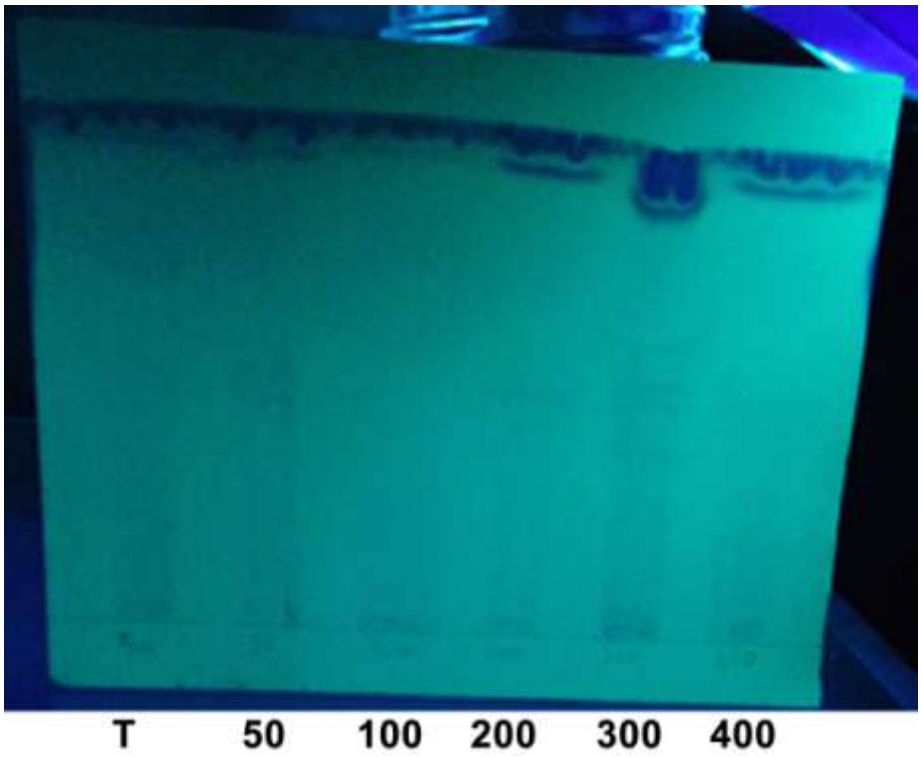


Figure 26: Photo of the plate after development with CuSO4 II, showing the migration of different phospholipids (Photo of Oluwagbemiga Amos S., 2015).

Daily administration of different doses of the aqueous extract of the bark of Sclerocarya birrea rats batches treated with the extract compared to the control group for 14 days influenced phospholipids in cells. Figures 26; 27 and 28 show the pictures of the plate obtained UV 260 nm respectively, UV 340 nm and after revelation with CuSO4 II after migration in a mixture: Chloroform / methanol / ammonia (32.5: 12.5: 2) until to 1 cm from the edge of the plate. Indeed this migration has effectively separate the different phospholipids. The first deposit corresponds to the negative control and the other are respectively the doses of 50mg / kg, 100mg / kg, 200mg / kg, 300mg / kg and 400mg / kg of aqueous bark extract Sclerocarya birrea. Considering the phospholipids in their entirety shows that:

- For the control there are about five spots so five phospholipids.
- For doses of 50mg / kg, 100mg / kg, 200mg / kg, 300mg / kg and 400mg / kg was also about five spots so five phospholipids.

However considering each lipid, note that:

- The spots at the witness and doses of 50mg / kg and 100mg / kg are very small, so each of the phospholipids in question is small amount compared to other higher doses.
- The spots at doses of 200mg / kg, 300mg / kg and 400mg / kg are sharper, darker compared to the control and to the first two doses so the amount of each of the phospholipids at these doses is greater by compared to the control and all other lower doses.

Furthermore, we note that at each submission it has more heavy fat y (down spots) only minor lipids (high in sp

V. DISCUSSION

The extraction yield is 9.1%. This result shows that the dry peel Sclerocarya trunk birrea contain active ingredients which are soluble in water, therefore extractable by the latter. In addition, the yield is low compared to that of Attakpa et al (16%) [32] and Ivonne Gisèle Makom Ndifossap et al (16%) [33], who conducted the aqueous extraction by maceration. These differences could be explained either by the extraction methods or by either solvents.

The administration of aqueous extract of the bark of Sclerocarya birrea at various doses: 50-400mg / kg shows on the photos 23, 24, 25 there is an increase in neutral lipids in cells having undergone the action of S. birrea large quantities: 200-400mg / kg than in the cells of the negative control and rats having undergone the action at low doses (50-100mg / kg); and this increase is proportional to the dose of S. birrea administered. So

we can deduce that S. birrea stimulates either the neutral lipid biosynthesis process or be neutral lipids are from degradation of membrane phospholipids.

- Each neutral lipid biosynthesis of processes emphasize the stimulation of several voice signaling, the synthesis of several enzymes and therefore the transcription of several genes. The idea that the S. birrea molecule alone can trigger all these processes seems unlikely. But these processes could be triggered by the presence of lesions in the membrane to compensate for losses of phospholipids.
- In the phospholipids biosynthesis process, neutral lipid (mono-, di- and tri- glycerides) serve as precursors [8; 34]. So conversely, in case of degradation of the phospholipids could have release neutral lipids.

Thus, the increase of neutral lipids through the action of S. birrea come from degradation of membrane phospholipids.

In addition, administration of the aqueous extract of the bark of S. birrea the 50 and 100mg / kg doses shown in photos 23, 24 and 25 there is no significant change in the neutral lipids profile compared the negative control. So in doses 50 and 100 mg / Kg there can be no degradation of phospholipids.

Photos 26, 27 and 28 show the action of the aqueous extract of the bark of Sclerocarya birrea at various doses: 50-400mg / kg of phospholipids. We see that there an increase in phospholipids in doses of 200,300 and 400 mg / kg compared to the negative control, while in doses 50 and 100 mg / kg there was no significant change compared to the negative control. Phospholipids are specifically membrane molecules, their strong presence in the lysates under the action of Sclerocarya birrea compared to the negative control may be explained by the fact that even before cell lysis by extraction solvents extract Sclerocarya birrea had induced the lysis of the plasma membrane; and this lysis is nothing but the result of necrosis. This result allows us to understand that the degradation of membrane lipids cited above is actually the result of necrosis.

VI. CONCLUSION

Our study was to assess the action of the aqueous extract of the bark of this plant on the lipid profile of rat hepatocytes. At the end of this study, it appears that in large doses: 200mg / Kg; 300mg / kg and 400mg / kg Sclerocarya birrea LEAD hepatocyte necrosis and thus is toxic. Furthermore this work allows us to circumvent this toxicity; because under the 50mg dose / kg and 100 mg / kg lipid profile did not change significantly compared to the negative control. So the aqueous extract administered Sclerocarya birrea be toxic at doses greater than or equal to 200mg / Kg.

These results suggest a great hope for the future as they will advise traditional practitioners with respect to the administration of doses for Sclerocarya birrea

treating patients to prevent the occurrence of liver disease. We believe that our results are significant or remarkable as they open for future experimental perspectives.

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