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Action of Ethyl Extracts of KhayaSenegalensis on Rat Hepatocytes. Evaluation of Toxicity

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ABSTRACT

The present work aims to study the effect of ethyl extract stem bark of Khayasenegalensis on the lipid profile of rat hepatocytes. For this, a sub-chronic gavage was performed on batches of 8 Wistar rats received 3 respectively, by oral administration of the extract at doses 2.5 mg / kg; 5mg / Kg; 10mg / Kg; 25mg / Kg; 50mg / Kg; 100mg / kg and 200mg / kg body weight for 14 days (control group received instead of the extract of Khayasenegalensis of distilled water) and isolation of hepatocytes was made after killing rats. Then, all the lipids (phospholipids and neutral) were extracted and separated by thin layer chromatography on two different plates. TLC of lipids (phospholipids and neutral) revealed an appearance of membrane lipids in the dose of 5 mg / kg; 10mg / Kg; 25mg / Kg; 50mg / Kg; 100mg / kg and 200mg / kg compared to control, while in the dose of 2.5 mg / kg there was no significant change compared to the control. The appearance of neutral lipids is only degradation of the phospholipids and the phospholipids provides that Khayasenegalensis lysed membrane of hepatocytes, which leads to the dispersion of the phospholipids. So it was only after dispersion, these lipids have started to deteriorate in neutral lipids. Where the administration of ethyl extract of trunk bark khayasenegalensis high dose may cause necrosis of liver cells, thus at high doses is toxic Khayasenegalensis. And the most efficient to mitigate the risk of toxicity are those doses less than or equal to 2.5 mg / Kg.

Keywords: Khayasenegalensis, phospholipids, neutral lipids, CCM, necrosis.

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INTRODUCTION

Africa and Benin in particular, there are many plants that are known for their therapeutic qualities, among them are: Khayasenegalensis and Azadirachtaindica (Meliaceae), Allium sativum L. and Aloe vera L. (Liliaceae) Anogeisusleiocarpus (Guill and perr.) and Combretummicranthum (G. Don) (Combretaceae) Ageratum conyzoides L. (Asteraceae), Eucalyptus globulus (Myrtaceae); etc. Khayasenegalensis is one of six species of the genus Khaya, native to tropical Africa and belonging to the family Meliaceae. It grows in many countries Togo, Cameroon, Mali, Cote d'Ivoire, Senegal [1, 2]. It is used in the treatment of conditions such as gastrointestinal disorders, enteralgia, inflammation, intestinal parasites, he also intervene in cardiac disorders (tachycardia, sore heart), vascular disease (varicose veins, hemorrhoids), skin problems (scabies, eczema), gynecological disorders (dysmenorrhea, infertility), the neuropathology (headache, hemiplegia), liver disease, diabetes, etc. [3, 4, 5]. Indeed, the use of K. senegalensis in the treatment of all those pathologies highlights several different functional mechanisms of the body's organs. In the case of diabetes, for example, organs are arraigned pancreas, liver, kidneys. But the liver is the heaviest and most voluminous organ in the body. It is located in the abdominal cavity in humans and housed in the right upper quadrant. It comprises 65% of liver cells (hepatocytes) that through their multiple functions, the liver put in interaction with other organs. Accordingly, any malfunction of the liver can have serious repercussions on many body functions. For several decades, diabetes is a disease that has plagued the world by its frequency and its complications. According to WHO, the prevalence is for a growth of about 35% [4] and after the screening of Boyle and his collaborators, the worldwide diabetic population would reach 300 million or more by 2050[6]. Given the complications and difficulties of the therapeutic management of diabetes and given the rumors K. senegalensis in the treatment of diabetes by traditional healers, many scientists have studied this plant to know his or her principle (s) active (s) and its mode of action to confirm or deny rumors of a hand and relieve the sick in the health and financial secondly plans. Thus, the laboratory Biomembranes and Cell Signaling did not remain indifferent to this state of affairs, it is a mission to find the mode of action of this plant to relieve the pains of diabetics worldwide and Benin in particular. Thus TAKIN et al (2013) showed on normoglycemic and hyperglycemic rabbits K. senegalensis can keep blood sugar normal in the first and can be back to normal in the second following the dose and time of administration[4]. These results give the K. senegalensis "hypoglycemic effects" and "anti hyperglycemic." In addition, TAKIN et al (2013) have shown that in the presence of K. senegalensis, isolated rat liver (in vitro assay) was observed movement

of glucose through. These results suggest that K. senegalensis could stimulate the storage of glucose in the liver. Thus these data gave rise to several questions such as: Khaya fact that the membrane of hepatocytes? Would he membrane receptors? How does it trigger the storage of glucose? It borrows the same signaling pathway as insulin? Khaya would it not toxic? All these questions then follows the subject of our work to assess the potential toxicity of Khayasenegalensis on rat hepatocytes to promote, encourage the use of this plant and eventually replace inconvenient conventional treatments such as insulin injection, or where appropriate discourage its use.

MATERIALS AND METHODS

Materials

Plant material

The bark of *Khayasenegalensis*, were collected in April 2011 in rain season. The plant material wasair-dried and milled into powder. The percolated mixture was filtered and evaporated with rotavapor

Animal

Wistarrat liver is use and subjected to a sub-chronic feeding (14 days) and were obtained from the Animal Breeding ofcytogenetics laboratory of the ISBA / FSS / Cotonou. 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

Preparation of extract from the bark of Khayasenegalensis

To obtain the extract ethyl bark of Khayasenegalensis, 200 g powder obtained, weighed using an analytical balance Sartorius® were macerated in 2000 ml of ethanol at 90 ° C under agitation continuously for 72 hours. Then, the macerate was filtered through hydrophilic cotton fiber. The obtained filtrate was evaporated at 40 ° C using the evaporator and the Rotavapor® rotatitif mesh is reused for a second maceration to increase yield. The paste deposited at the bottom of the evaporator flask was recovered in jars and put in oven-drying at 45 ° C. After complete drying, the solids at the bottom multiples jars were scraped using a spatula stainless steel, crushed in a mortar porcelain and kept in glass bottles previously labeled. Finally, the yield is calculated.

Feeding rats

In this operation, it is administered the ethyl bark extracts KhayasenegalensisWistar rats (males and females) and approximately 200g of the same diet, and orally at various doses for about two weeks. These animals received no other medication in the time outside of the extract. The rats

were randomly divided into 08 lots, 03 rats (No. 1, No. 2 and No. 3) and attributed respectively to lots doses per kg body weight per day: 0 mg / Kg; 2.5 mg / kg; 5 mg / kg; 10 mg / kg; 25 mg / kg; 50 mg / kg; 100 mg / kg and 200 mg / kg; and sets the volume to be administered at V = 01 ml. Then, each rat weighs each lot in order to find the average weight and the effective dose of extract which will be administered is calculated. Gave then daily and at the same time the rats for 14 days. Lot 1 is the control group and received oral administration of distilled water instead of the extract ethyl (0 mg / kg) of Khayasenegalensis for 14 days; other lots (2-8) are treated by oral administration, the respective doses of 2.5 mg / kg; 5 mg / kg; 10 mg / kg; 25 mg / kg; 50 mg / kg; 100 mg / kg and 200 mg / kg body weight per day of the extract of Khayasenegalensis ethyl, for 14 days; Finally, the rats were sacrificed 24 hours after the last gavage, to take our livers to various manipulations.

Isolation of rat hepatocytes using the cold trypsinization[7, 8, 9, 10, 11].

The trypsinization is the method for breaking the tissue to isolate cells using the exposure to hot or cold method. Due to tryptic activity of trypsin in the method of trypsinization hot at 37°C; the tissue is damaged and harvesting the cells after 30 minutes of incubation may lead to cell damage or reaggregation. These inconveniences can be overcome by using a minimum concentration of trypsin and a long incubation time in order to increase the separation efficiency of trypsin. At longer incubation times, the tryptic activity affects the cells and therefore the temperature should be minimized to reduce the triptych effect. A simple method to reduce damage to cells during exposure is to soak the fabric with trypsin at 4 ° C for 6-18 h to allow penetration of the enzyme with low tryptic activity followed by exposure of 20 to 30 minutes at 37°C for disintegration. Rats (250 g) were anesthetized by inhalation of chloroform, and the liver was isolated by a horizontal cut on the ventral surface near the diaphragm of the animal. The isolation of the liver time does not last more than 2 to 3 min. The liver was maintained in phosphate buffer saline solution (PBS) at 4 ° C until use. The experimental environment was sterilized. 0.375 g of liver was removed and then cut with the surgical blade into small pieces; these pieces were washed with PBS, and then transferred to Petri dishes and other unwanted tissues such as adipose 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 after the operation of 10 ml per 1 g of tissue. After incubation, the liver pieces were exposed at 37°C for 30 minutes in 1 ml per 100 mg of warm RPMI tissue. In order to completely disperse the tissue is made gentle pipetting, then the solution is sterile filtered through a muslin cloth to remove the larger pieces. Cells were preserved for experiments and stored in common crop in 5% dimethyl

sulfoxide (DMSO) as cryoprotectant used, added to the cell to reduce the formation of ice and thereby prevent cell death in during the freezing process, and the cells may be frozen at -80°C and stored in liquid nitrogen in complete safety).

Extraction and thin layer chromatography of cellular lipids

Lipid extraction

The lipid extraction method is based on the method known as solvent gradient used by Stephan et al. (2004) for extracting mycobacterial lipids. It has been adapted to the hepatocytes. Four successive extractions are carried out on the same sample cell. The extraction solvent is methanol / chloroform increasingly enriched in chloroform [12, 13]. The cellular lipids were extracted by four successive extractions a day, with stirring at 250 rpm. The lipid extract is recovered after separation of the cells; adding an aqueous solution of 0.9% NaCl (CHCl3 proportional to volume) afforded the two phases [14, 15]. The cells are then ready to undergo the next extraction. The proportion of solvents chloroform (CHCl3) and methanol (CH3OH) are different according to the extraction step:

First extraction: CHCl3 / CH3OH (1V: 2V)

Second extraction: CHCl3 / CH3OH (1V: 1V)

Third extraction: CHCl3 / CH3OH (2V: 1V)

Fourth extraction: CHCl3 / CH3OH (2V: 1V)

A volume V = 15 mg per 500 ml of cells

All the organic phases are combined and the solvent was evaporated under a hood or Rotavapor. The lipid extract is resuspended in a volume of chloroform (1 ml of solvent to 10 mg of lipids). Then the lipid extract is stored at -80 ° C or used for the analysis.

Note: If the sample was stored at - 80 ° C, it must be evaporated under a stream of nitrogen and then resuspend in chloroform to know the precise volume before lipid analysis [15].

Thin layer chromatography (TLC) of cellular lipids

This is the separation of different classes of lipids on silica gel thin layer chromatography. CCM is based on electrostatic interaction / hydrogen bond. The principle of "like attracts like that," often encountered in chemistry allows still here to explain the nature of the phenomena involved. It is based on the differential adsorption phenomena: a mobile phase consisting of solvent progressing along a stationary phase (silica) on a fixed plate. TLC takes place in three stages: preparation of the tank, preparation of the plate, and elution. The various cellular lipids are divided into two major groups: phospholipids and neutral lipids. They are separated on two different plates. [13]

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, free sterols and esterified. They are separated using low-polar solvents: hexane, ethyl ether.The separation of neutral lipids is first by migration in a hexane solvent: diethyl ether: glacial acetic acid (80: 20: 2 v / v / v) up to half of the plate. The plate is then dried a second migration is performed until 1 cm from the upper edge of the plate with hexane alone [13, 15].

Preparation of the vessel (mobile phase)

Eluent E1: it corresponds to the M1 mixture of the following solvents: hexane: ethyl ether: glacial acetic acid (80: 20: 2 v / v / v) [15, 13].

Eluent E2: it is the only hexane [13].

The two eluents are placed in two different vessels C1 and C2 at a height of about 0.5 cm from the bottom of the tank; then the tanks are closed so that they are saturated eluent steam for 10 to 15 min.

Preparation of stationary phase

On a silica plate (Silica gel 60, Merck, France) with dimensions of 10 × 10 cm, draw a pencil, a line about 1 cm from the bottom edge and parallel to it; is then deposited on the line, using capillary tubes and 0.5 to 1 cm apart, 10 mL of each sample to be analyzed lipids (control, sample 1, sample 2 ...) [15, 13].

Elution or development of the chromatogram

The plate is placed in a slightly oblique position in the cell C1 is then closed. The solvent which covers the bottom rises along the plate by capillary action. Migration is terminated when the solvent front reaches about half of the plate. The plate is then placed in the second tank C2 and migration after drying is stopped when the solvent front reaches the top of about 1cm from the plate. The level reached by the solvent (front line) is indicated by a thin line and the plate is dried in the open air or using a drier [15, 13].

Separation of phospholipids by TLC

Phospholipids are polar molecules separated using combinations of highly polar solvent: water, methanol, chloroform ... Migration was once with a mixture: chloroform / acetone / methanol / acetic acid / H2O (50/15/10 / 10/4 (v / v) or chloroform / methanol / glacial acetic acid / water (25: 15: 4: 2) or chloroform / methanol / ammonia (65: 25: 4). these proportions are on the verge of . two-phase mixture must remain monophasic [15, 13].

Preparation of the vessel (mobile phase)

The eluent corresponds to the mixture of the following solvents: chloroform / acetone / methanol / glacial acetic acid / H2O (50/15/10/10/4) or chloroform / methanol / glacial acetic acid / water (25: 15: 4: 2) or chloroform / methanol / ammonia (65: 25: 4). The eluent was placed in a tank at a height C3 of about 0.5 cm from the bottom of the tank; then the vessel is closed so that it is saturated with steam for eluting 10-15 min [15,13].

Preparation of stationary phase

On a silica plate (Silica gel 60, Merck, France) with dimensions of 10 × 10 cm, draw a pencil, a line about 1 cm from the bottom edge and parallel to it; is then deposited on the line, using a capillary tube and 0.5 to 1 cm apart, 10 mL of each sample to be analyzed lipids (control, sample 1, sample 2)

Elution or development of the chromatogram

The plate is placed in a slightly oblique position C3 in the vessel is then closed. The solvent which covers the bottom rises along the plate by capillary action. Migration is terminated when the solvent front reaches approximately 1 cm from the upper end of the plate. The level reached by the solvent (front line) is indicated by a thin line and the plate is dried in the open air or using a drier [15, 13].

Revelation of lipids on TLC plates

Revelation is made by spraying a solution of copper II sulfate 10% (10 g in 100 mL of CuSO4, $5H_2O$ 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 [13].

RESULTS AND DISCUSSION

Extraction yield

The yield is calculated using the formula published by Takin et al

Table 1: Table showing the yield obtained after extraction

Extract	Performance in%	Color	Aspect
Macerated / ethanol	16,6	Red wine powder	Powder

Action of ethyl bark extract of K. senegalensis on the profile of neutral lipids in rat hepatocytes

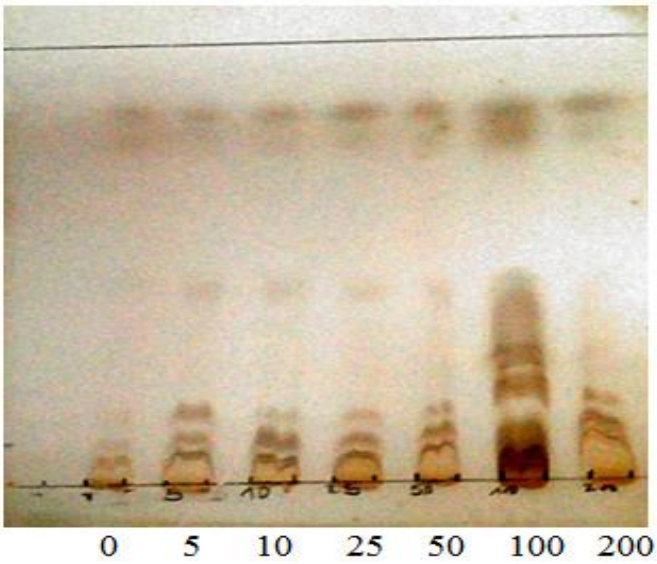


Figure 1:Photo of the plate obtained after migration of different neutral lipids

In groups of rats treated with the extract, it was found that the daily administration of different doses of the extract ethyl Khayasenegalensis bark compared to the control group for 14 days influenced qualitatively and quantitatively different types of lipids cells in neutral. Figure 1 shows the picture of the plate obtained after a first migration with hexane / ethyl 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 5 mg / kg, 10mg / kg, 25mg / kg and 50mg / kg 100mg / kg and 200mg / kg of bark extract ethyl K. senegalensis. Whereas in the neutral lipids in their entirety is found that:

For the control there are four spots about four neutral lipids

For the doses of 5mg / kg, 10mg / kg, 25mg / kg and 50mg / kg was more than six to six spots, therefore neutral lipids.

For doses of 100mg / kg and 200mg / kg was more than seven spots longer than seven neutral lipids.

Considering each lipid, we note that:

The spots at the witness are very low, so each lipid in question is a small amount compared to other The spots at doses of 5mg / kg, 10mg / kg, 25mg / kg and 50mg / Kg least low compared to the control, so the amount of each of the lipids in these doses is greater relative to witness. Spots at doses of 100mg / kg and 200mg / kg are sharper, darker compared to the control, so the amount of lipids in each of these doses is greater compared to the control and all other doses. In addition, it is noted that at each there is more deposit heavy lipids (spots bottom) that slight lipids (spots from

above). Including the dose of 2.5 mg / kg of extract, we see that there is no significant change in the dose for this compared to negative control lipid profile. Indeed, at the witness as to the level of 2.5 mg / kg dose was two weak spots, so there are two neutral lipids in small quantities. As at doses of 5mg / kg and 10mg / kg was well visible four spots, so the presence of four lipids in large quantities compared to the control and 2.5 mg / Kg.

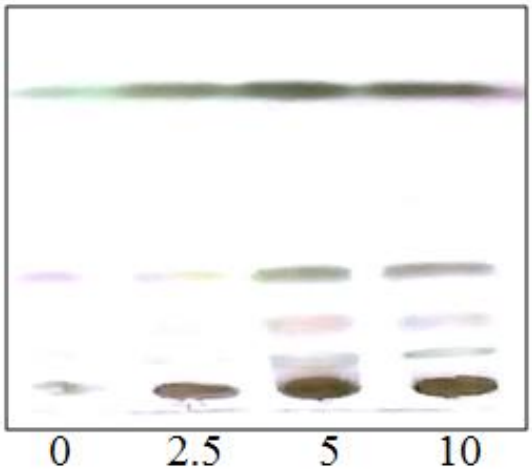
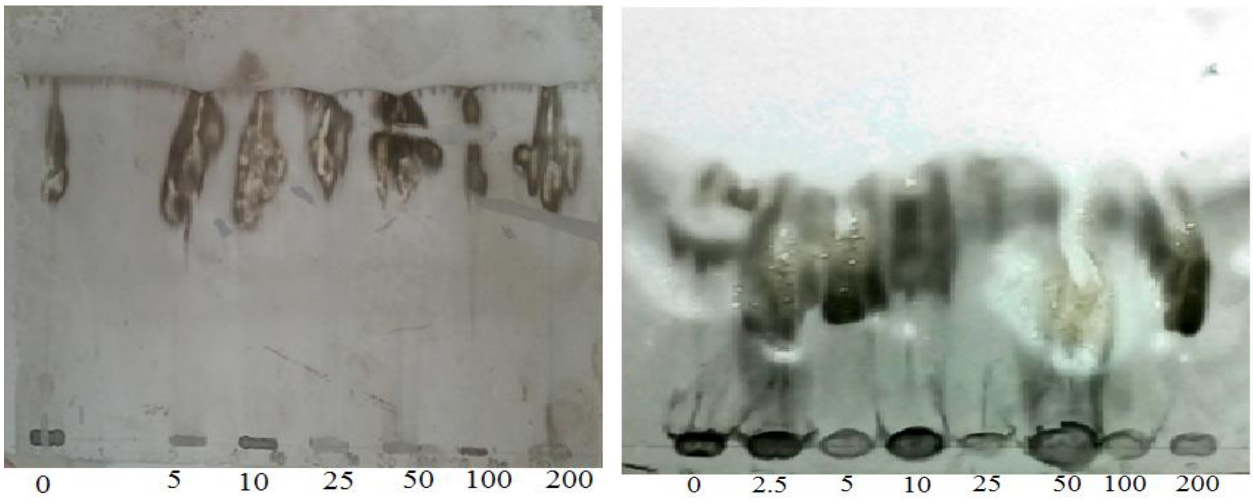


Figure 2:Photo of the plate obtained after migration of different neutral lipids.

The first deposit corresponds to the negative control and the others are respectively doses 2.5 mg / kg; 5mg / Kg; 10mg / kg, ethyl extract from the bark of Khayasenegalensis

Action of ethyl extract of the bark of Khayasenegalensis on the profile of phospholipids in rat hepatocytes



(a) (b)

Figure 3: Photograph of the plates obtained after migration of different phospholipids with a mixture (a) chloroform / acetone / methanol / glacial acetic acid / water (50: 15: 10: 10: 5 v / v); (b) chloroform / methanol / glacial acetic acid / water (25: 15: 4: 2)

Daily administration of different doses of the extract of the bark of ethyl Khayasenegalensis in groups of rats treated with the extract relative to the control group during 14 days influenced phospholipids in cells. Figure 2 shows the plates obtained after migration with a mixture (a) chloroform / acetone / methanol / glacial acetic acid / water (50: 15: 10: 10: 5 v / v); (b) chloroform / methanol / glacial acetic acid / water (25: 15: 4: 2) to 1 cm from the edge of the plate; This allows to separate the various phospholipids. The first deposit corresponds to the negative control and the others are respectively doses 2.5 mg / kg, 5mg / kg, 10mg / kg, 25mg / kg and 50mg / kg 100mg / kg and 200mg / kg of ethyl extract of the bark of Khayasenegalensis. Indeed, different eluent used do not allow to see the separation of different groups of phospholipids; but it is noted that according to the eluent, phospholipids form a cluster of spots on the one hand, which migrates to the solvent front (a) and are on the other hand dragged (b). In addition, we note that these clusters are larger spots at doses 5mg / kg, 10mg / kg, 25mg / kg and 50mg / kg 100mg / kg and 200mg / kg compared to the negative control and the 2.5 mg / Kg dose which are smaller. Phospholipids are therefore low in quantity at the negative control and 2.5 mg / kg dose when they are in large quantity at doses 5 mg / kg, 10mg / kg, 25mg / kg and 50mg / kg 100mg / Kg and 200mg / Kg. The barks of Khayasenegalensis used are those collected by the team in 2011 and used by TAKIN et al. The yield of 16.6% obtained after extraction is low compared to that of TAKIN et al (22.62%). This could be explained by the conditions and shelf life of bark on the one hand; and the concentration of ethanol was used as the other. The administration of ethyl extract of Khayasenegalensis bark at various doses: 5-200mg / Kg Figure 1 shows the picture that there is an increase in neutral lipids in cells that have undergone the action of K. senegalensis that in negative control cells; and this increase is proportional to the dose administered K. senegalensis. So we can infer that K. senegalensis is stimulates the process of biosynthesis of neutral lipids or be neutral lipids are derived from the degradation of membrane phospholipids. The process of each neutral lipid biosynthesis highlights stimulating several voice signaling, the synthesis of several enzymes and thus the transcription of several genes. The idea that the molecule can K. senegalensis has itself initiated this whole process seems unlikely. But these processes could be triggered by the presence of lesions in the membrane to compensate for losses of phospholipids. In the process of biosynthesis of phospholipids, neutral lipids (mono, di and tri glycerides) serve as precursors [16, 14]. Inversely in the case of phospholipid degradation it would release neutral lipids. Thus, the increase of neutral lipids by the action of Khayasenegalensis come from the degradation of membrane phospholipids. In addition, administration of the extract ethyl bark of Khayasenegalensis the dose 2.5mg / kg 20 shows the picture that there is no significant change in the profile of neutral lipids

by negative control report. So in the 2.5mg / kg dose there may be degradation of phospholipids. Figure 3 shows the action of ethyl extract of Khayasenegalensis bark at various doses: 2.5; 5-200mg / Kg on phospholipids. It is noted that there yardstick increased phospholipids in doses 5-200mg / kg compared to the negative control, while in the 2.5mg / kg dose there is no significant change compared to the negative control. Phospholipids are molecules specifically membrane, their strong presence in lysates under the action of K. senegalensis compared to the negative control could be explained by the fact that even before cell lysis by extraction solvents the K. senegalensis extract had induced the lysis of the plasma membrane; lysis and this is nothing other than the result of necrosis. This result allows us to understand that the degradation of membrane lipids cited above is actually the result of necrosis. Our results are consistent with the results of Adébayo et al (2003) showed in their study on some biochemical parameters of rat kidneys, the administration of the extract ethyl Khayasenegalensis (2 mg / kg body weight) for 18 days causing hypernatremia due to the diffusion of Na + from the pool in the environment and also a decrease in alkaline phosphatase (ALP), an enzyme "label" is often used to assess the integrity of the plasma membrane and endoplasmic reticulum. According to these authors attributed this reduction would be the loss of membrane components (including the alkaline phosphatase) or in extracellular fluids is to inhibition of the enzyme or its inactivation by the in situ sample; therefore there would be necrosis of tissue from the body is that the contact with foreign substances including the extract [17]. Similarly, our results are consistent with those of UN et al (2013) have shown in their research on the effect of aqueous extract of the bark of Khayasenegalensis on some biochemical and hematological parameters and histopathological rats that following administration of doses of 400, 800, 1200, 1600 and 2000 mg / kg of extract for 28 days, they also observed a significant increase in biochemical and hematological parameters Na +, K +, creatinine, ALT, AST, ALP and a significant reduction in the total protein concentration. These authors conclude that have hypernatremia and hypercalcemia are due to a failure or renal dysfunction, resulting in poor control of the balance of electrolytes by the kidneys. The significant increase of aminotransferases shows lysis of hepatocytes and that of the enzyme "marker" ALP confirms the disruption of the plasma membrane of the liver (necrosis). As regards the total protein, it is said that in case of very extensive damage in the liver, their concentration is reduced entirely. [15]In addition, our results allow us to confirm the renal or muscle cell damage particularly in the liver, heart, suspected by Takin et al (2013) after dosing kidney and liver enzymes ALT, AST, GGT and G6PDH on the one hand and histological studies of the liver and kidneys on the other hand. Indeed these authors have shown that following administration of the extract of Khayasenegalensis ethyl was a significant

increase of enzymes ALT, AST and GGT respectively greater than or equal to 2.5 mg / kg doses, 1 mg / kg 1mg / kg body weight; or generally, the extension of the lesion is directly proportional to the increase of the activity of these enzymes. They also noted a significant reduction in G6PDH and some changes in the hepatic tissue (connective tissue accumulation, expansion of the space of Disse, appearance of pyknotic nuclei in hepatocytes) and kidney tissue (glomerular abnormalities, tubular dilation) in doses of 10mg, 25 and 50mg / kg body weight; then they inferred that there is a correlation between the decrease of tissue damage and G6PDH [18]. Moreover, other authors have shown that the problem of toxicity caused by the ethyl Khayasenegalensis extract in high doses could be corrected by a synergy with cinnamon (work in process of publication). So there is a similarity between these results wholes; when the administration of the extract ethyl Khayasenegalensis (greater than 2.5 mg / kg body weight doses) is prolonged and repeated the integrity of underlying tissues (liver, kidney, heart) and their function is compromised. Recall that the necrosis is a "pathological judgment, abnormal functioning of a cell." It was the first form of cell death highlighted. It comes after a severe attack of the cell. It is not determined by intrinsic factors, but only by environmental disturbances. It is the result of extreme non-physiological conditions and is not engaged in a "voluntary". It is a passive, rapid and non-specific mechanism. It can be induced by various factors such as the lack of oxygen supply (hypoxia, ischemia), physical agents (mechanical trauma, thermal, radiation), chemical and infectious (viruses, bacteria, fungi, parasites), reactions immunological, nutritional imbalances. Unlike apoptosis (programmed cell death, necrosis is considered a "disorderly" cell death. Early loss of ion flow control at the membrane causes massive and excessive penetration of the water and later, the lysis of the plasma membrane. This leads to the release of cytoplasmic contents into the surrounding medium. Organelles tend to swell. The permeability of the inner and outer mitochondrial membranes is modified by increasing the concentration of calcium in these organelles. Lysosomes will break, releasing the cytoplasm of hydrolytic enzymes that contribute to the destruction of the cell. Nuclear DNA is degraded in a "random" endonuclease activated by serine proteases including [19].

CONCLUSIONS

In Africa and Benin in particular, people are resorting to traditional medicine for health needs. For decades, *Khayasenegalensis* is used in the treatment of several diseases and even diabetes is a scourge. Our study was to evaluate the action of ethyl bark extract of this plant on the lipid profile of rat hepatocytes. At the end of this study, it appears that in high doses: 5 mg / kg; 10mg / Kg; 25mg / Kg; 50mg / Kg; 100mg / kg and 200mg / kg of KhayaSenegalensis causes necrosis of

hepatocytes and hence is toxic. Moreover, this work allows us to circumvent this toxicity; because in the 2.5mg / kg dose lipid profile there was no significant change from the negative. These results suggest a great hope for the future as they will advise traditional healers compared to administration doses of Khayasenegalensis for the treatment of patients to prevent the development of liver disease. Our results are remarkable for us as they open for future experimental perspectives.

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