VOLUME-7, ISSUE-1, JANUARY-2018 • PRINT ISSN No 2277 - 8160

Original Research Paper

Pathology

ANNONA MURICATA REDUCED PROLIFERATION *CAUSED BY CYCAS REVOLUTA* IN WISTAR RATS

Sêmassa M. I. HOTEYI	Laboratory of Biomembranes and signalling Cell University of Abomey Calavi/Benin
Omedine KOUKOUI	Laboratory of Biomembranes and signalling Cell University of Abomey Calavi/Benin
Loetitia MICHODJEHOUN	Laboratory of Biomembranes and signalling Cell University of Abomey Calavi/Benin
Natacha AIVODJI	Laboratory of Biomembranes and signalling Cell University of Abomey Calavi/Benin
Eudoxie K. ASSOU	Laboratory of Biomembranes and signalling Cell University of Abomey Calavi/Benin
Alphonse SEZAN*	Laboratory of Biomembranes and signalling Cell University of Abomey Calavi/Benin. *Corresponding Author

ABSTRACT

The purpose of this work is to determine the phytochemic composition of the ethyl extracts of the sheets of *Annona muricata*, in order to check of them the antineoplasm effects (anti-cancer) on cancer of the liver to the Benin.For this fact, an ethyl extraction using powder of sheets dried of *Annona muricata* and ethanol 90° was initially carried out. The screening phytochimic of the sheets of the plant was carried out by the method of Hougton and Raman. And finally the antineoplasm activity (anti-cancer) was evaluated by dosage of the parameters ALAT, ASAT and proteins total on grundings livers of Wistars rats, at the cancer of the liver was armature as a preliminary by exposure to the powder of *Cycas revoluta* through a diet composed to 5% of this powder and a drink water containing an amount de10mg/kg powder of *Cycas revoluta*.

The results of the screening revealed the presence of several chemical compounds equipped with various properties to varied concentrations. The identified chemical compounds are: reducing compounds, leuco-anthocyanes, tanins, flavonoïdes, coumarins, mucilages and triterpenes. The antineoplasm activity (anti-cancer) was effective by administration of an amount of 100 mg/kg of ethyl extracts of the sheets of *Annona muricata*. This activity showed a variation of intensity according to the mode and the duration of administration of the extracts

KEYWORDS : Annona muricata, Cycas revoluta, cancer of the liver, antineoplasm, extracted, Benin

INTRODUCTION

T he data collected over several years reinforces the idea that cancer is a major concern in developing countries (Dcs), as in the rest of the world in the coming years. (1) According to the World Health Organization (WHO), the incidence of cancer is increasing worldwide. In 2000, 5.3 million men and 4.7 million women around the world have developed cancer and 6.2 million people died. Cancer was, at that date, responsible for 12.5% of adult deaths worldwide, more than the proportion of deaths from HIV, tuberculosis and malaria combined. According to the World Cancer Report, published in 2003 by the IARC (International agency for research on cancer), cancer incidence could rise by 50% in the next twenty years, with 20 million new cases per year of 2020 and 10 million dead in the absence of a global mobilization. (1)

In fact, cancer is a group of diseases characterized by increased and uncontrolled and abnormal proliferation of cells in the body. (2) The disruption of the cell operation, which is the origin of cancer, is due to the intervention of exogenous factors. Thus, according to WHO, among the factors that can promote the occurrence of cancer are: low consumption of fruits and vegetables, alcohol and tobacco and also chronic viral infections. Note that there are several types of cancer, one of the most frequent in Africa liver cancer. This cancer usually occurs as a result of liver disease. (3) According to the WHO 70 percent of all liver cancer deaths occurred in developing countries that focus mainly in Africa. (4)

Africa, has always made use of plants in almost all areas. Indeed, human life on earth is closely related to the operation of the plants. These have the capacity to produce wide range of natural substances. Besides the primary metabolites, they often accumulate so-called secondary metabolites that are an important source of molecules used by man in particular in the pharmacological field. The plants are recognized as a wonderful source of drugs. (5,6) It is thus that the drug management of chronic conditions say (such as liver cancer) is largely ensured by the use of medicinal and food plants. Hence the importance of herbal medicine for African people with very low income. (4.7) It is in this context that the extracts of Annona Muricata, commonly called soursop are used by tradihérapeutes in the West African region, particularly in Benin in the treatment of liver cancer.

Indeed soursop, Annona muricata is a small tree of the family Annonaceae, native to northern South America, it is cultivated in tropical regions (8); particularly in West Africa (Ivory Coast, Senegal, Benin, Togo) for its edible fruit, and its use as antimicrobial painkillers in joint diseases and cancer. In Benin it is commonly called "Chaps-Chaps".

So our work is the study of Annona Muricata, which is a plant widely used in cancer pharmacopoeia in Benin. Its almost systematic use by herbalists in treatments of liver cancer, so have pushed us to understand the mechanisms of action both pharmacological phytochemical plan. Thus, we can confirm or refute the traditional uses of Annona Muricata in liver cancer treatment and contribute to development and local production of traditional medicines defined toxicity and effectiveness and low cost.

I-3 Conventional treatment of liver cancer and Short comings

Traditional treatment of liver cancer that are chemotherapy, radiation and surgery are not always effective. (27) Indeed partial hepatectomy is reserved for tumors less than 5 cm in diameter in patients with cirrhosis Child A. Alcohol, or destruction acétisation the radio frequency in these small tumors produce results equivalents. Indeed, recurrence is common because of intrahepatic metastasis unknown or new cancer foci. Liver transplantation, only truly curative treatment is only useful for very small tumors (risk of recurrence in the other cases). Medical means are palliative. Chemoembolization can sometimes cause a partial or complete tumor necrosis, but its benefit in terms of survival is not demonstrated. There is no general chemotherapy or hormone useful. Moreover, these treatments cause unbearable side effects and are virtually inaccessible to people for many reasons, and here are a few:

- The Technical facilities and health facilities are inadequate. Health workers often lack of competence, either in terms of diagnosis or patient care.
- The high cost of cancer (ATC).
- And Finally unavailability, lack of insurance financial access to medicines by social security is a serious handicap in Africa.
- Thus, in developing countries, more than half of the population living in rural areas and uses a lot of medicinal plants that offer their traditional healers for the treatment of liver cancer. Among these plants used in the traditional treatment of liver cancer was the Annona muricata, which is the object of our study.

General information on Annona muricata

a)

b)

c)

Figure 5. Photos of the leaves, flowers, fruit and the tree of Annona muricata

(*Photo AÏVODJI Natasha. 2014*)

To mitigate the side effects of cancer treatment, scientists research in several plants such as Annona muricata, used in traditional African pharmacopoeia, (28) including Benin. Soursop is exclusively native to the tropical forests: Caribbean, Central and South America. It is also common in other parts of the world with a tropical climate, such as sub-Saharan Africa, including Benin. Many industries mainly exploit its fruit but also other parts of the plants. In traditional medicine Benin decoctions of the leaves of Annona muricata are commonly used as a sedative, diuretic, and to treat influenza and dysentery. Furthermore it used as anti hypertensive, anti-inflammatory, anticancer, and as insect repellent.

Systematic position of Annona muricata

kingdom:
Plantae Order: Magnoliales
Subkingdom:
Tracheobionta Family: Annonaceae
Division:
Magnoliophyta Genre: Annona
class:
Magnoliopsida Binomial name: Annona muricata
Subclass:
Magnoliidae

Biology of Annona muricata

Soursop (Annona muricata) is a shrub or small tree from March to October m high. (8) leaves, bright green, are oblong-lanceolate, 10-17 x 2-7 cm, young ferruginous pubescence below. Flowers appear on large pedicels (15-20 mm long) opposite to the leaves. 6 petals are yellow, fleshy and thick. The three outer petals are broadly ovate, close to edges without overlapping. It blooms all year. It tolerates poor soils but does not support the low temperatures

The very large fruit is formed by the meeting of the carpel (a syncarpous). It is 15 to 20 cm long, even up to 30 cm long. Dark green, it is covered with a soft curved areolas outgrowth. The white pulp is edible and contains black seeds flattened. In Benin soursop typically found in the south, is a fruiting tree, domesticated, it is often grown in urban (Cotonou, Porto-Novo), in the courtyards of houses. However it is also found in the northern savannas.

MATERIALS AND METHODS

Plant Material

The plant material of our study is made of the leaves of Annona muricata and Cycas revoluta.

Biological Materials

The biological material consists of liver Wistar rats.
Laboratory equipment
Extraction

- The plant material consists of the fresh leaves of Annona muricata
- Distilled water
- Tooth grinder
- 90 ° ethanol
- Device ROTA VAPOR
- incubator
- Analytical Balance Sartorius

II-3-2- Phytochemical Screening

- Hydrochloric acid
- Mayer Reagent
- Ether chloroform
- Anhydrous disodium sulphate
- Distilled water
- Chloroform
- Reagent Stiasny
- Acetate disodium
- Ferric Chloride
- Magnesium powder
- Ammonia
- Reagent Shinoda
- Acid dinitobenzoique
- Sodium hydroxide
- Alcoholic solution 1% dinitrobenzene
- Acetic acid
- Acetic sulfuric acid Anhydrous

II-3-3- searches anticancer effects of the extracts ethyl leaves of Annona muricata

- **Reagents**

- Normal Saline
- Acid-washed sand (beach sand washed in 35% sulfuric acid)
- BIOLABO kits (ALT, AST, and total protein)
- Sterile distilled water

- **Equipments**
- Dissection kit
- Probe
- Eppendorf tubes
- Mortar
- Centrifuge
- glass
- Spectrophotometer
- Micropipettes
- Blades
- Balance

METHODS

Extraction

Dryings leaves

Fresh leaves of Annona muricata were harvested in May and in September 2013 as a family plot in the city of Cotonou (district Akpakpa). They were identified by botanists experts from the National Herbarium of the University of Abomey Calavi (Appendix 1) and dried in the shade on a bench of Biomembranes Laboratory, Cell Signaling and size at the Faculty of Sciences and Technology (FAST), University of Abomey Calavi (UAC) for four weeks for the leaves picked in May and in the oven at 55 ° C LBTMM (Laboratory of Microbiology and Molecular Typing) size also FAST UAC; for leaves picked in September. After drying Dried leaves are ground and made into powder using a brand RETSCH knife mill and stored in glass jars to prevent the installation of microorganisms pollutants.

Figure 6. Powder of dried leaves of Annona muricata (Photo Natacha AIVODJI. 2014)

Preparation of total extracts from the leaves of Annona muricata

Extraction themselves, was done in the laboratory of Pharmacognosy and essential oils to the size ISBA (Cotonou).

We took so 100g powder leaves picked in September after grinding have seen these leaves that we provided more powder; that was macerated in 1 liter of ethanol for 72 hours with stirring. After maceration evaporated ethanol at 40 ° C using the evaporator, rotary evaporator. The recovered extracts were placed in an oven at 45 ° C for drying. Then the dried extracts were scraped. The dried extracts were weighed and calculated efficiency.

The extract solution which has served for the following experiments was prepared with distilled water. The extract thus obtained in the form of a powder, was used for the various studies conducted.

Figure 7. Extraction Process on leaves of Annona muricata (a: Weight of the powder of the leaves; b: powder mixed in ethanol 90 ° with stirring; d: filtrate mixture leaf powder / ethanol 90 ° evaporation the ROTA VAPOR d: extracts from the leaves of Annona muricata dried and crushed) (Photo Natacha AIVODJI 2014).

III-2 Screening Phytochemical

The Phytochemical Screening is based on the reactions (color and precipitation) differential of the main groups of chemical compounds in the plants according to the method of HOUGHTON PJ and A. RAMAN (1998) reviewed and adapted to the conditions of Pharmacognosy Laboratory and Essential Oils .

III-3 Research anticancer effects of ethyl extracts from the leaves of Annona muricata

We used 15 adult Wistar rats of both sexes, which we divided into 5 lots of 3 rats.

III-3-1-Checking liver cancer induction powder cycads

Cycas revoluta is an ornamental plant of the family of cycads, which some studies have carcinogenic effects.

Thus in order to use the powder of the leaves to induce liver cancer necessary for our study, we verified the alleged carcinogenic effects. We therefore initially, worked with two groups of rats on 5 lots planned for our study. One office had witnesses and received only simple distilled along with a diet comprising granules water and tap water for 15 days; while the second batch it was receiving a supply of compound granules and 5% of powder of Cycas revoluta and 10mg / kg of powder diluted cycads in drinking water.

At the end of 15 days the rats were sacrificed by chloroform anesthesia and dissected.

After dissection, the livers of the rats were collected, crushed; and this ground has been the measurement of parameters such as: ALT, AST and total protein.

- **Determination of parameters: ALT, AST, and total protein**

To assay the various parameters that are ALT, AST and total protein, liver tissue samples and weighing 0.5 g, previously rinsed in saline; were crushed in a mortar with 5 ml of a normal saline solution and the washing acid sand for 10 minutes at room temperature. Then crushed livers were centrifuged for 5 minutes at 6000 turn. The recovered supernatant served in the spectrophotometric assay of the activity of ALT, AST and total protein.

Pharmacological Activities of ethyl extracts from the leaves of Annona muricata After verification of the carcinogenic activity of the powder leaves of Cycas revoluta, we took the last 3 groups of rats, which are numbered from 1 to 3. It was therefore respectively Lot 1 witnesses rats not taking as distilled water for the entire duration of the experiment, then the batch 2 which rats were fed with diet containing pellets and 5% of powder of Cycas revoluta and 10mg / kg diluted in cycads powder drinking water, accompanied by a gavage of 100mg / kg weight ethyl extracts from the leaves of Annona muricata and this during the five weeks of experimentation, and finally the lot 3, the rats had during the first 2 weeks testing the compounds diet pellets and 5% of powder of Cycas revoluta and 10mg / kg diluted cycads powder in the drinking water. Then in the last 3 weeks they were force-fed with a solution of 100mg / kg weight ethyl extracts from the leaves of Annona muricata dissolved in distilled water together with a diet without powder Cycas revoluta . Note that the rats received daily at 1 ml of the extract solution; administered using an esophageal probe.

After 5 weeks of experiment, the rats were sacrificed and then dissected. After dissection, the livers of the rats were collected, crushed; and this ground has been the measurement of parameters such as: ALT, AST and total protein.

RESULTS AND DISCUSSIONS

Rendements de l'extraction

Soit R le rendement de l'extrait,

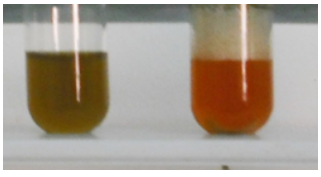
$$R = \left(\frac{Masse\ de\ l'extrait}{Masse\ de\ la\ poudre\ de\ feuilles}\right) \times 100$$

AN : R= $R = \left(\frac{5,1}{50}\right) \times 100$

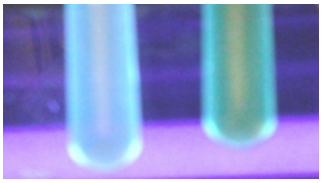
R=10,2%

L'extraction a donné un rendement moyen de (10.2%)

a)



b)



c)

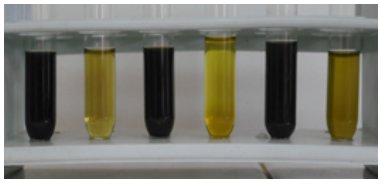


Figure 9. Some results of the identification of chemical compounds (a: test flavonoids; b: test coumarins; c: test tannins; TT: Tube witness TP: Test positif

- Absent
+ Slightly Abundant
++ Abundant

We have abundant reducing compounds.
We also tannins, catechin tannins, gallic tannins, leuco anthocyanin whose chemical group of polyphenolic compounds that are moderately abundant in the extract of Annona muricata.

We also find in our extracts compounds such as mucilage, flavonoids, triterpenoids and also the coumarins in our extracts.

Table IV: Results of phytochemical screening

Composés phytochimiques	Présence
Mucilages	+
Composés réducteurs	++
Dérivés cyanogéniques	-
Tanins simples	+
Tanins catéchiques	+
Tanins galliques	+
Flavonoides	+ (flavones)
Anthocyanes	-
Leuco-anthocyanes	+
Alcaloïdes	-
Dérivés quinoniques	-
Saponosides	-
Triterpénoïdes	+
Stéroïdes	-
Cardénolides	-
Coumarines	+
Anthracéniques libres	-
O-hétérosides	-
O-hétérosides à réduites	-
C-hétérosides	-

Checking the carcinogenic effect of the powder Cycas

The administration of powder Cycas revoluta for 2 weeks, or 15 days significantly influenced the change in parameters ALT, AST, and total protein.

Indeed, it is observed compared to control rats that received during the experiment as distilled together with a single diet water comprising pellets and tap water; ASAT growth rate and total protein; and a decrease in the rate of ALT in rats given a diet with 5% powdered Cycas revoluta in food (pellets) rats and 10mg powder Cycas revoluta per kg body weight of rats in water beverage

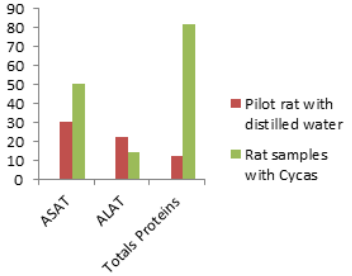


Figure 10. Histogram showing the variation parameters of ALT, AST, and total protein in a diet containing or not containing the powder Cycas revolute

Checking the antiproliferative activity of the extracts ethyl leaves of Annona muricata.

The results of the comparison of parameters ALT levels, AST and total protein by the type of treatment received by the rats show a fairly significant influence in the administration of ethyl extracts from the leaves of Annona muricata.

Indeed, it is observed that the administration of powder Cycas revoluta rats batch ps (ps: stimulated proliferation) after the first experimental phase evokes abnormal hepatocyte proliferation of these rats through a growth rate ASAT and total protein, with a decrease in ALT levels in these rats.

Then it is observed compared to the batch ps, rats that received Lot 2 as a treatment for 5 weeks experimental diet containing the powder cycads at doses described above and a daily gavage using our extracts at a dose of 100mg / kg body weight of rats; we experienced a decline in their rate of AST and total protein and an increase in ALT levels.

Finally, we observe that compared to batch ps, the rats of Lot 3 as receiving treatment during the first 2 weeks of experimental diet containing the powder cycads at doses described above then daily gavage using our extracts at a dose of 100mg / kg body weight of rats during the last 3 weeks together with a diet without powder Cycas revoluta; we experienced a decline in their rate of AST and total protein and increased ALT levels more significantly than observed in rats of lot 2.

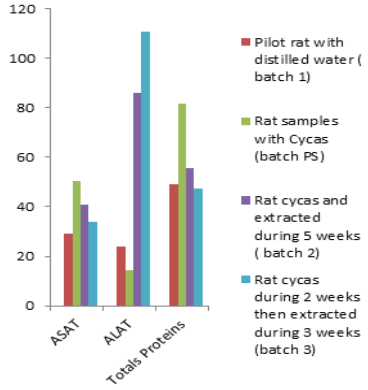


Figure 11. *Histogram showing the variations parameters of ALT, AST, and total protein in a diet containing or not containing the powder Cycas revoluta and administration of ethyl extracts from the leaves of Annona muricata*

Cancer is a group of diseases characterized by increased and uncontrolled and abnormal proliferation of cells in the body. (2)

This is one of the main causes of death. Also the number of people with continued growth in cancer. (24) In fact every year 2 or 3% of all deaths worldwide result from the different types of cancers. (35)

The high cost of conventional treatment methods available including surgery, chemotherapy, and radiation therapy (27); and the lack of effective drugs to treat tumors encourage populations in different countries, especially in sub-Saharan Africa, where one of the most common cancers are liver cancer; to rely more on traditional medicine, which is based on the use of medicinal plants. (36)
These plants have an almost unlimited ability to produce substances of interest to the researchers involved in the fight against cancer through the development of new anti-cancer treatments. (24) In an extensive study, the anti-cancer properties of 187 plant species were evaluated (37) including Annona muricata.

Annona muricata (Annonaceae) commonly known as "Chap-chap" is widely used in traditional medicine in Benin to treat liver cancer. Studies in Nigeria Ngozi Okolie by P. et al. have shown an anti-cancer effect of ethyl extracts from the leaves of Annona muricata in colorectal cancer induced by Cycas circinalis is a plant of the same family as Cycas revoluta.

Indeed, this study conducted in Nigeria has shown that a dose of 100 mg / kg body weight of rats of ethyl extracts from the leaves of Annona muricata, for 3 weeks, preceded by a cancer induction consumption compound feed 5% of the powder Cycas circinalis; induce a decrease of cell proliferation. It would attribute this anti-proliferative activity in the acetogenins which are compounds in Annona muricata. The acetogenins destroy cancer cells by the complex lock-CoQ oxidoreductase Nadh (complex 1), which provides them ATP. The acetogenins also will possess effective anti oxidant properties. (24)

From these data, we set out to assess the antiproliferative activity of ethyl extracts from the leaves of Annona muricata.

So we conducted an ethyl extraction using the powder of the leaves of Annona muricata and ethanol 90 °. We after this extraction obtained a 10.2% yield, we deem means compared to the yield obtained by Eka Prasati et al. (2012), which is 14.86%. This difference is explained by the difference in degree of Ethanol which is 70 ° to them.

We then made a phytochemical screening with the method of Houghton and Raman (1998), which allowed us to detect the following compounds: gearboxes, tannin compounds, catechin tannins, gallic tannins, leuco anthocyanin whose group chemical polyphenolic compounds, mucilage, flavonoids, and triterpenoids. These results are partially in compliance with those of Vijayameena C. et al. (2013) have identified two other groups of compounds that are more alkaloids and saponins and using the method described by Harborne et al. (1973). (39) This difference is explained by the difference method and also the origin of the plant used to make the extract.

Cycas revoluta contains cycasin (methyl azoxymethanol aglycone), which is known to induce liver cancer and many others 28. After ingestion of a meal containing powder Cycas, intestinal bacteria hydrolyze the glucoside link cycasin to release the aglycone methyl azoxymethanol. (24)

Since researchers have realized effective carcinogenic methyl azoxymethanol, these agents have been used to create reliable cancer animal models (to induce cancers). (40) With this in mind, we have also used the powder of the leaves of Cycas revoluta, a plant of the same family as Cycas circinalis found in Benin, to induce cancer in our study.

So we have to do this, first check in the carcinogenicity of powdered leaves of Cycas revoluta. So we took 6 Wistar rats divided into 2 groups of 3 rats each. One office had witnesses and received only simple distilled along with a diet comprising granules water and tap water for 15 days; while the second batch it was receiving a supply of compound granules and 5% of powder of Cycas revoluta and 10mg / kg of powder diluted cycads in drinking water.

At the end of 15 days the rats were sacrificed by chloroform anesthesia and dissected. After dissection, the livers of the rats were collected, crushed; and this ground has been the measurement of parameters such as: ALT, AST and total protein. It must be stressed that the parameters that are to be assayed ALT and AST were chosen because as cytosolic enzymes hepatocytes, the variation in the rate in the outcome of a liver homogenate piece may reflect an increase in the number of cells, thus cell proliferation. Regarding total proteins, which are the result of the transcription of certain genes, their sudden increase may reflect an abundant and abnormal transcription and carcinogenesis could be characterized by the body concerned (the liver).

We obtained the result of this experiment, as compared to control rats that received during the experiment as distilled water accompanied by a single diet comprising pellets and tap water; ASAT growth rate and total protein; and a decrease in the rate of ALT in rats given a diet with 5% powdered Cycas revoluta in food (pellets) rats and 10mg powder Cycas revoluta per kg body weight of rats in water beverage. This is consistent with the results of Ngozi Okolie P. et al. (2013).

This increase in the rate parameters AST and total protein in rats that consumed the powder of Cycas revoluta be explained by a greater production of succinate end of the Krebs cycle that is the logical continuation of glycolysis and is in addition known as intermediate between mitochondrial dysfunction seen in cancer cells and oncogenesis via the HIF-α factor. It augur abnormal liver hepatocyte proliferation of these rats; This allows us to test the hypothesis of the carcinogenicity of the powder leaves of Cycas revoluta.

After this verification we searched the antiproliferative activity of ethyl leaf extracts Annona muricata. For this we took the three groups of rats, which were numbered from 1 to 3. So we had lot 1 respectively witnesses rats took only distilled water along with a diet without powder Cycas revoluta, throughout the duration of the experiment, then the batch 2 which rats were fed a diet consisting of granules and 5% of powder of Cycas revoluta and 10mg / kg diluted cycads powder in water beverage together with a gavage of 100mg / kg of weight of ethyl extract of the leaves of Annona muricata and during the 5 week experiment, and finally the batch 3, the rats were during the first 2 weeks of the experiment the compounds of pellet diet and 5% of powder of Cycas revoluta and 10mg / kg of powder diluted cycads in drinking water. Then in the last 3 weeks they were force-fed with a solution of 100mg / kg weight ethyl extracts from the leaves of Annona muricata dissolved in distilled water together with a diet without powder Cycas revoluta .

The results showed that the administration of powder Cycas revoluta rats batch ps (ps: stimulated proliferation) after the first experimental phase evokes abnormal hepatocyte proliferation of these rats through a growth rate ASAT and total protein, with a decrease in ALT levels in these rats.

Then it was observed that compared to batch ps, rats that received Lot 2 as a treatment for 5 weeks experimental diet containing the powder cycads at doses described above and a daily gavage using our extracts in a dose of 100mg / kg body weight of rats; experienced a decrease in their rate of AST and total protein and an increase in ALT levels.

Finally, it has been found that compared to the batch ps, the rats of Lot 3 as receiving treatment during the first 2 weeks of experimental diet containing the powder cycads at doses described above then daily gavage using our extracts at a dose of 100mg / kg body weight of rats during the last 3 weeks together with a diet without powder Cycas revoluta; experienced a decrease in their rate of AST and total protein and increased ALT levels much more significant than observed in rats of lot 2.

This decrease in AST levels and total protein, accompanied by the growth of ALT levels in rats treated simultaneously Lot 2 to the powder Cycas and our extracts, and lot 3 treated powder Cycas and our extracts allowed us to say that the administration of ethyl extracts from the leaves of Annona muricata reduced the abnormal proliferation of hepatocytes induced by powdered leaves of Cycas revoluta with our rats, and is in agreement with the results of Okolie N'Gozi P . et al. (2013). Hence the hypothesis of an antiproliferative activity of the extracts from the leaves of Annona muricata is confirmed.

Finally, unlike the effects of leaf extracts of Annona muricata as they were administered simultaneously with or after powder Cycas Cycas powder could be explained by the fact that the acetogenins would anticancer active compounds of our extracts mode of action to eliminate cancer cells already induced. (24)

And the extracts are more effective when administered after the induction of proliferation by the powder of Cycas.

This low efficiency in the case of the simultaneous administration of our extracts with the powder of Cycas could also be explained by the fact that the active compounds in these plants might have the same receptors on cells, but would trigger channels contrary signs. Thus simultaneity in the administration of the two plants could induce competition, where would this low conspicuity of the effect of our extracts in this case.

REFERENCES

1- Laurine Lavergne (2008). L'accès aux médicaments anticancéreux dans les pays en développement d'Afrique subsaharienne : l'exemple de Madagascar, RESEAU MEDICAMENTS & DEVELOPPEMENT (ReMeD) N°38
2- Karp G. (1999). Cell and Molecular Biology (second edition), Wiley, USA
3- Wang Y, Xu K, Lin L, Pan Y, Zheng X. (2007). Geranyl flavonoids from the leaves of Artocarpusaltilis. Phytochem. 68: 1300-1306.
4- Abiodun, Falodun, Bjames Osakue, Duzoekwe Anayo Stephen et Ciu Sheng-Xiang (2011). Phytochemical And Anticancer Studies On Ten Medicinal Plants Used In Nigeria, Bayero Journal Of Pure And Applied Sciences, 4(1): 36 – 39
5- Bénédicte Akakpo (2012). Action d'extraits éthyliques de Khaya senegalensis sur quelques paramètres biochimiques chez le lapin. Incidence sur l'histologie du foie et des reins des lapins, p1
6- ZOHOUN Loïca, (2011). Contribution à l'étude hypoglycémiante des extraits de Khaya senegalensis (étude in vitro sur le foie du rat blanc de laboratoire) p. 2
7- Geoffrey, C., Kirby, M. (1996). Medicinal plants and the Control of Protozoa disease with reference to Malaria. Transaction of the Royal Society of Tropical Medicine and Hygiene,London, vol 3. Pp 605-609.
8- Jacques Fournet (2002). Flore illustrée des phanérogames de Guadeloupe et de Martinique, Gondwana editions, Cirad, (ISBN 2-87614-489-1 (Cirad, Tome 1). - 2-87614-492-1 (Cirad, Tome 2).)
9- Edah, K. M. (1999). Etude des traitements des cas de cancer admis au CNHU de Cotonou à propos de 452 cas recensés du 1er janvier 1987 au 31 décembre 1996 , 153 p.
10- Meeks, R., Harrison, S. et Bull, R. (1991). Hepatotoxieology, CRC Press., FL
11- O'Grady, J., Lake, J. et Howdle, P. (2001). Comprehensive clinical hepatology, Mosby, St. Louis
12- Jones, A. et Spring-Mills, E. (1983). The Liver and Gal!bladder, Elsevier Ed., L. Weiss, New York
13- Campra, 1. et Reynolds, R. (1989). The Hepatic Circulation, Raven, New York
14- Muto, M. et Yamamoto, M.(1978).The Liver: An Atlas of Scanning Electron Microscopy., Igaku-Shoin, Tokyo
15- Loud, A. V. (1968). «A quantitative stereological description of the ultrastructure of normal rat liver parenchymal cells». J Cel! Biol. vol. 37, no l, p.27-46.
16- Bennett, A. L., Paulson, K. E., Miller, R. E. et Darnell, 1. E., Jr. (1987). «Acquisition of antigens characteristic of adult pericentral hepatocytes by differentiating fetal hepatoblasts in vitro». J Cel! Biol. vol. 105, no 3, p. 10731085.
17- Berry, M. N. et Friend, D. S. (1969). «High-yield preparation of isolated rat liver parenchymal cells: a biochemical and fine structural study». J Cel! Biol. vol. 43, no 3, p. 506-520.
18- Belinsky, S. A., Badr, M. Z., Kauffman, F. C. et Thurman, R. G. (1986). «Mechanism of hepatotoxicity in periportal regions of the liver lobule due to allyl alcohol: studies on thiols and energy status». J Pharmaeol Exp Ther. vol. 238, no 3, p. 1132-1137.
19- Haussinger, D. (1990). «Nitrogen metabolism in liver: structural and functional organization and physiological relevance». Biochem 1. vol. 267, no 2, p. 281-290
20- Wilson, 1. W. et Leduc, E. (1948). «The occurrence and formation of binucleate cells and polypoid nuclei in the mouse». liver Am. 1. Anat. vol. 82, p. 353-391.
21- Gebhardt, R. (1988). «Different proliferative activity in vitro of periportal and perivenous hepatocytes». Scand J Gastroenterol Suppl. vol. 151, p. 8-18.
22- Guguen-Guillouzo, C., Clement, 8., Baffet, G., Beaumont, c., Morel-Chany, E., Glaise, D. et Guillouzo, A. (1983). «Maintenance and reversibility of active albumin secretion by adult rat hepatocytes co-cultured with another liver epithelial cel1 type». Exp Cell Res. vol. 143, no 1, p. 47-54.
23- Gaasbeek Janzen, 1. W., Gebhardt, R., ten Voorde, G. H., Lamers, W. H., Charles, R. et Moorrnan, A. F. (1987). «Heterogeneous distribution of glutamine synthetase during rat liver development». J Histochem Cytochem. vol. 35, no 1, p. 49-54.
24- Wamidh Hadi Talib. (2011). Anticancer and Antimicrobial Potential of Plant-Derived Natural Products, Phytochemicals – Bioactivities and Impact on Health
25- Jean-Loup Rey. (2008). Comment prévenir les cancers dans les pays en développement ?, RESEAU MEDICAMENTS & DEVELOPPEMENT (ReMeD) N°38
26- SNFGE. (1999). Cancer du foie ?, Réponses issues des objectifs nationaux rédigés par le Collège des enseignants d'hépato-gastroentérologie.
27- Tannock, F. (1998). Conventional cancer therapy: promise broken or promise delayed?.Lancet, 352, 9-16.
28- Anil Kumar. (2012). A Review On Hepatoprotective Herbal Drugs, International Journal Of Research In Pharmacy And Chemistry, Ijrpc, 2(1)
29- R.J. et al. (1960). Revised spectrophotometric methods for detrmination of GOT, GP and LDH, Am. J. Clin. Path., 34, 381-397
30- H.U. Bergmeyer et al. (1978). Optimization of methods for Aspartate aminotransferase and Alanine amino transferase, clin.chem.24: 58-73
31- IFCC. (1986) .Expert Panel on Enzymes part 3, .Jclin.chem.Biochem.,24:481-495.
32- M. Mathieu et al. (1978.). SFBC comité de standardisation.Recommandation pour la mesure de la concentration catalytique de l'alanine aminotransferarse dans le sérum à 30°C , Ann.Biol. Clin. 36. 457-462
33- Gornall A. C., Bardawill C. J., David M. (1949), J.Biol. Chem. 177, 751
34- Weischselbaum, TE. (1946), J. Clin. Path, 16,40
35- Madhuri, S., & Pandey, G. (2009). some anticancer medicinal plants of foreign origin. Current Science, 96 (6), 779-782.
36- Wood-Sheldon, J., Balick, M. & Laird, S. (1997). Medicinal Plants: Can Utilization and Conservation Coexist?. The New York Botanical Garden. USA.
37- Kintzios, E. (2006). Terrestrial plant-derived anticancer agents and plant species used

in anticancer research. Critical Reviews in Plant Science, 25, 79–113. International Journal of Applied Science and Technology Vol. 2 No. 1; January 2012 157
38- Eka Prasasti ,Nur Rachmani, Tuti Sri Suhesti Retno & Widiastuti Aditiyono (2012). The breast of anticancer from leaf extract of annona muricata againts cell line in t47d. International Journal of Applied Science and Technology Vol. 2 No. 1; January 2012
39- C. Vijayameena, G. Subhashini, M. Loganayagi and B. Ramesh. (2013). Phytochemical screening and assessment of antibacterial activity for the bioactive compounds in Annona muricata. Int.J.Curr.Microbiol.App.Sci 2(1): 1-8
40- Laqueur G.L. (1965). The induction of intestinal neoplasmin rats with cycasin and its aglycone. Virchows Arch Ab A Pathol Anat; 340: 151-153
41- Ministère de la santé publique du Bénin, 2010. Annuaire des statistiques sanitaires de 2010 en République du Bénin. PP.73-105
42- www.janvier-labs.com
43- Yves Delange, (2009). « Les Cycadales sur quatre continents », Hommes & Plantes, vol. 70,