# ***Original Research Article***

# *In vivo antifibrotic potential of extracts of Acanthospermum hispidum DC. evaluated in wistar rats using diethylnitrosamine*

.


## ABSTRACT

**Aims:** Liver fibrosis is a chronic disease of the liver. This disease is a stage of passage to liver cancer. The objective of this work was to evaluate the ability of the ethanolic extract of *Acanthospermum hispidum* to block the progression of hepatic fibrosis induced in rats using diethylenitrosamine (DEN).

**Study design:**Study of the antifibrotic potential of extracts of *Acanthospermum hispidum.*

**Place and Duration of Study:***In vivo* tests were performed from September 2018 to January 2019. The animal model tests were carried out in the pet shop of the Institute for Health Sciences Research (IRSS) of Burkina Faso and in the Cytogenetics Laboratory (FSS/ISBA) of the Republic of Benin.

**Methodology:**The evaluation of the antifibrotic activity consisted in treating in *wistar* rats a liver fibrosis induced with the DEN which is a chemical agent whose effect on the liver has already been confirmed. As a result of the treatment, all animals were removed from the liver and blood. The livers were used for macroscopic and microscopic observations. Blood has been used for the evaluation of biochemical parameters in relation to fibrosis.

**Results:**The analysis of the results of the biochemical parameters in relation to the fibrosis showed that the ethanolic extract of *Acanthospermum hispidum* at the dose of 250 mg / kg made it possible to obtain an improvement of these parameters compared to the other batches of animals. These results have been confirmed by those of the anatomo-pathological studies.

**Conclusion:**The results of biochemical and histological analyzes revealed a capacity of *Acanthospermum hispidum* extracts to block the evolution of hepatic fibrosis in the rat. These results confirm the hepatoprotective potential of this medicinal plant used in traditional medicine in Burkina Faso.



*Keywords: Acanthospermum hispidum*, Diethylnitrosamine, liver, *in vivo* antifibrotic


## 1. INTRODUCTION

Hepatic fibrosis is due to the excessive accumulation of matrix components in the liver. In addition to the quantitative increase in collagen and other matrix proteins, it is characterized by qualitative changes in the nature of the matrix components deposited and their distribution in the liver [1]. Hepatic fibrosis complicates all chronic liver diseases, whether due to chronic alcoholism, viral B or C infection, or autoimmune, biliary, parasitic or medicinal. It is now accepted that hepatic fibrosis is a dynamic process, causing not only excessive production of matrix components (fibrogenesis) [2], but also a decrease in their degradation (fibrolysis) [3].

The WHO estimates that 2 billion people are infected with the hepatitis B virus and 400 million have chronic carriers, including 60 million in Africa [4]. Burkina Faso has an estimated

prevalence of 14.4% of the hepatitis B virus [5]. Viral hepatitis, in particular those caused by hepatitis B and C viruses, cause respectively 1300 and 900 deaths from liver cancer each year, which they can cause [6]. The latter constitutes in Burkina Faso the first cause of health evacuations out of the country and the third cause of mortality after infectious diseases and cardiovascular diseases [7][8]. Treatment of chronic hepatitis in Burkina Faso could cost $ 909 per month per patient [5]. The fibrosing diseases represent 45% of the causes of mortality in the world. The lack of therapeutic alternatives for the management of patients with chronic hepatitis makes liver fibrosis a very poor prognosis [9].

*Acanthospermum hispidum* is an herb selected from an ethnobotanical survey that identified the medicinal plants used in Burkina Faso's traditional medicine against liver diseases and had a good anti-hepatotoxic capacity *in vivo*. The objective of this study was to evaluate the ability of the ethanolic extract of *Acanthospermum hispidum* to block the progression of hepatic fibrosis induced in experimental animals. For this purpose, diethylnitrosamine (DEN), a toxic substance known to induce hepatic fibrosis in laboratory animals, has been used as a hepatotoxin.

## 2. MATERIAL AND METHODS

### 1.1 Material

**Plant material**

The plant material consists of the whole plant (roots, stems and leaves) of *Acanthospermum hispidum* harvested The whole plant of *Acanthospermum hispidum* was harvested in September 2018 at Loumbila (12 ° 19'35.84 N, 1 ° 35'13.5 W). The plant has been identified at the Laboratory of Plant Ecology and Botany of University Ouaga I Pr Joseph KI-ZERBO.

**Consumables**

Aluminum foil, Kit surgery, 1cc and 5cc Syringes, porcelain mortar, blades and microscope slides, gloves, bleach, blotting paper, micropipettes, Eppendorff tubes, Alcohol 90. They are all analytical grade.

**Reagents**

Diphenylboryloxyethylamine, sodium phosphate monobasic ($NaH_2PO_4$), dibasic sodium phosphate ($Na_2HPO_4$), EDTA (Ethylenediaminetetraacetic acid), Diethylnitrosamine (DEN), Silymarin.

**Physiological Solutions**

Phosphate buffer, tris buffer, dimethylsulfoxide (DMSO), sodium hydroxide, sodium chloride (9 ‰), potassium chloride (9 ‰), formalin buffer (10%).

### 1.2 Methods

**2.2.1 Extraction by ethanol maceration**

The dry plant material has been made into powder using a grinder. Fifty grams (50 g) of the whole plant powder were extracted with stirring for 24 hours with 1000 mL of pure ethanol. After filtration under reduced pressure, the filtrate was frozen and freeze-dried.

**2.2.2 Animal treatment**

Pre-test allowed to identify doses of DEN to be administered to rats, as well as the duration of treatment required to obtain the liver fibrosis.

The antifibrotic activity in curative mode was evaluated according to the following protocol [10] with some modifications:

Male *Wistar* rats were randomly assigned to batches of eight (8) rats after a two-week acclimation period. The rats used were free of pathogenic organisms and healthy status. The experiments met the requirements of the Code of Ethics: The Institutional Animal Ethics Committee (Directive 2010/63 / EU on the protection of animals used for scientific purposes). Ethical approval code: 2010/63 / EU, Date of approval: 20 October 2010.

Group I (Normal Group): The rats received standard treatment during the eight weeks (a standard treatment is to give water and pellets of food to the animals at will).

Group II (Negative control group): The rats in the group received water in place of the extract after administering the DEN intraperitoneally (75 mg/kg body weight) once a week during the 4 first weeks.
Group III (Positive Control Group): Rats received a daily dose of 100 mg/kg silymarin (that means that, knowing the weight of the animal administered, we determine the mass of silymarin administered to this animal. Thus this mass is weighed and dissolved in water at a volume not exceeding the margin 5 to 20 ml/kg of weight) for 4 weeks after intraperitoneal injection of DEN (75 mg/kg body weight) once a week during the first four weeks.
Test Groups IV and V: The rats received intraperitoneally DEN (75 mg/kg of body weight per week) during the first four weeks and during the last four weeks these animals received daily doses (100 and 250 mg/kg body weight) of ethanolic extract of *Acanthospermum hispidum*.

**2.2.3 Registration of body weight of animals**

The body weight of the treated animals was recorded using a scale at 1$^{st}$ day, week 2, week 4, week 6 and week 8, and compared to animals from normal group (group II).

**2.2.4 Biological analyzes**

*2.2.4.1 Collection of blood and liver*

Animal blood was collected by cardiac puncture using a 5 mL syringe. To collect, it was first necessary to stabilize the heart using a pair of pliers. The sample was taken from the left ventricle. The collected blood had to reach at least a volume of 3 mL so that after centrifugation we can collect a sufficient volume of serum for the various analyzes. The collected blood was centrifuged at 3000 g for 10 minutes. After centrifugation, the clear (supernatant) serum was recovered using 1 mL syringes and placed in the cryotubes for biochemical markers analysis.

The livers of the animals were removed by getting rid of the stomach, diaphragm and adhesions. The livers were kept in formalin (10%) for the pathological study.

*2.2.4.2 Biochemical analyzes*

Blood samples were taken for biochemistry. These blood samples in the tubes without anticoagulant were centrifuged for 10 minutes to obtain serum. Serum has been used for the evaluation of biochemical parameters that are indirect markers of liver fibrosis such as aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), total bilirubin (bilirubin T), albumin and alkaline phosphatase (PAL). All these parameters were determined using kits (Selectra XL Vital Scientific Elitech Group Company) according to the instructions of the manufacturer.

**2.2.5 Histopathological analyzes**

The livers of the treated animals were removed, weighed and used for histological analysis. The methodology used was that of Hould [11]. Liver sections (about 0.2 × 0.2 cm) were made with the rotating microtome (Leitz 1512). These sections were fixed in 10% formalin and then placed in a paraffin bath. The liver slices were then labeled with hematoxylin-eosin. Finally, these labeled liver slices were subjected to microscopic examination for histological analysis.

**2.2.6Statistical analysis**

The data were expressed as mean ± standard deviation. Graphics were drawn and statistical analysis was performed using GraphPad Prism software version 5.0 for Mac OS X (GraphPad software, San Diego, California, USA).

## 3. RESULTS AND DISCUSSION

### 3.1. Evolution of biochemical parameters

For transaminase values, a very significant difference ($p < 0.001$) exists between ALAT and ASAT values in DEN-only and 250 mg-treated animals. kg of body weight. This finding was also made for the ALAT and ASAT values of animals treated with silymarin and those treated with the extract at a dose of 100 mg / kg body weight compared to the negative control (DEN). In addition, the ASAT value in animals treated with silymarin and in animals treated with the ethanolic extract of *Acanthospermum hispidum* at a dose of 250 mg / kg body weight, showed a significant difference ($0.05 > p > 0.01$) with respectively 148.00 ± 12.17 U/L and 119.67 ± 13.80 U/L.

Compared to alkaline phosphatase values, a very significant difference ($p < 0.001$) was observed between the mean value of DEN alone and those treated with ethanolic extract at 250 mg/kg body weight as well as for controls. Animals treated with the ethanolic extract at the dose of 250 mg/kg also showed a mean value of alkaline phosphatase which is not statistically different from that of the controls; which on the other hand is statistically very different ($p < 0.001$) from the value of animals treated with silymarin (100 mg/kg) with respectively 127.00 ± 17.52 U/L and 85.00 ± 14.42 U/L.

The values of albumin and bilirubin did not differ significantly between those treated with DEN alone and animals from other lots. In contrast, a low mean value of albumin was recorded in the animals that received only DEN (21.43 ± 0.76 g/L). The highest mean value of bilirubin was also observed in animals treated with DEN alone (8.47 ± 0.76 U/L) (Figure 1).

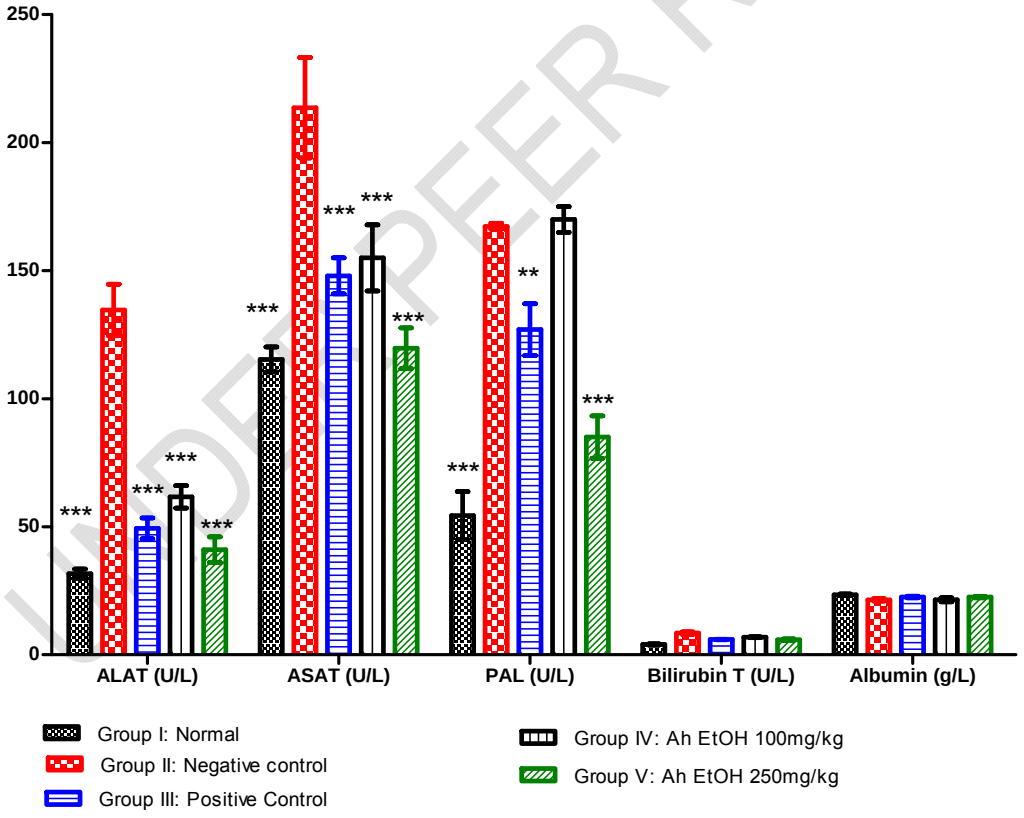



**Fig. 1.Results of Biochemical Parameters of Treated Animals**

*ALAT: Alanine Amino-transferase; ASAT: Aspartate Amino-Transferase; MDA: MalonDiAldehyde; PAL: Alkaline phosphatase: significant from positive control, * P < 0.05; ** P < 0.005; *** P < 0.001*

*Mean ± S.E.M = Mean values ± Standard error of means of eight experiments.*

### 3.2 Variation in animal weight and relative weight of livers

Animal weight analysis showed a highly significant difference (p> 0.001) between animals treated with ethanolic extract at 100 mg / kg and 250 mg / kg compared to those in group 2 (DEN alone) at the fourth week. By the eighth week, it appears that the difference between the average weight of the animals treated with the extract at the dose of 250 mg/kg and that of the animals treated with the DEN alone is very highly significant (p <0.001). In addition, there was a statistical difference (p> 0.01) between the mean weight of animals treated with silymarin and those treated with the extract at a dose of 250 mg/kg (Table 1).

**Table 1. Effect of the extracts on the variation of the weight of the animals**

| Groups | 1st day | 2nd week | 4th week | 6th week | 8th week |
|---|---|---|---|---|---|
| Group I | 165.33±3.78* | 189.33±13.69** | 209±12.43*** | 229.33±16.93*** | 235.67±14.87*** |
| Group II | 170.17±4.12 | 165.33±3.62 | 162.67±10.48 | 160.67±11.18 | 168.00±15.72 |
| Group III | 180.85±9.5* | 178.82±7.25** | 170.48±6.48** | 168.42±6.98* | 173.64±7.01** |
| Group IV | 174.83±3,60 | 1693.00±6.26* | 167.00±13.24** | 161.33±9.18 | 165.67±5.96* |
| Group V | 178.67±3.78 | 172.67±9.18* | 170.30±11.54** | 169.00±5.59* | 175.67±8.12*** |

*Group I: control, Group II: DEN, Group III: DEN + Silymarin, Group IV: DEN + Ah 100mg, Group V: DEN + Ah 250mg, significant from positive control, * P < 0.05; ** P < 0.005; *** P < 0.001Mean ± S.E.M = Mean values ± Standard error of means of eight experiments.)*

The relative weight values of the livers (The relative weight of the liver is determined by the ratio of the liver weight to the total weight of the animal multiplied by 100) of the treated animals did not show a statistical difference between the batches. On the other hand, the average values of the relative weight of the organs show that the relative weight of the livers of the negative control lot is relatively high compared to the other lots (Figure 2).

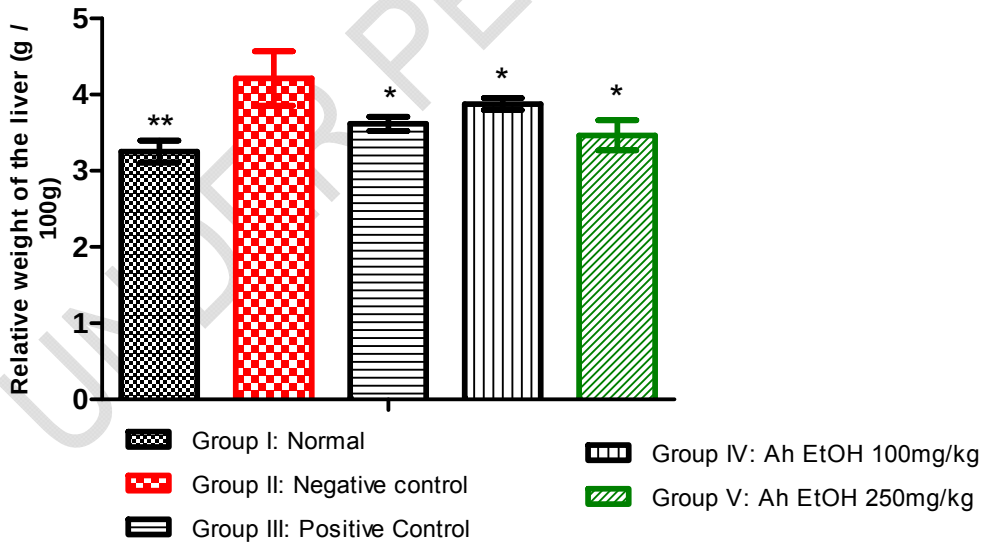



**Fig. 2. Effect of the extracts on the variation of the relative weight of the livers of the treated animals**

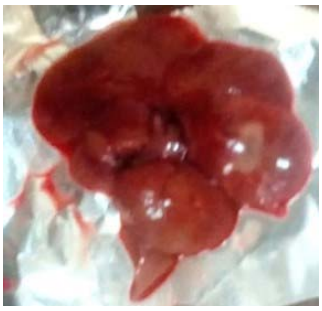
*significant from positive control, * P < 0.05; ** P < 0.005;*
*Mean ± S.E.M = Mean values ± Standard error of means of eight experiments.*

## 3.3 Histopathological studies

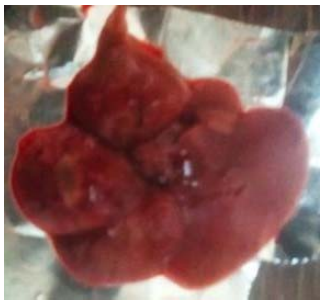
Macroscopic observation of the liver in treated animals showed that, compared to the liver of the animals in the control group (photos 1.a), that of the negative control group (photos 1.b) had a brownish or whitish coloration on its surface (nodules). In addition, compared to the negative control group, the animals treated with the extract at the dose of 250 mg/kg (photo 1.e) have livers whose state is significantly improved. Finally, compared to the liver of the animals in the positive control group (silymarin 100 mg/kg of body weight), the ethanolic extract at the dose of 250 mg/kg presented a liver with a more regular appearance.

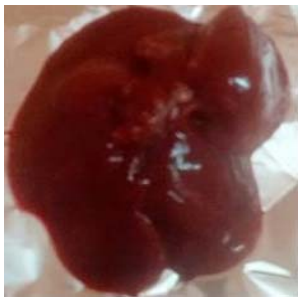
Microscopic observation of liver sections in normal control animals shows normal liver cells with a well preserved cytoplasm and a visible central vein. This shows the absence of collagen deposition on hepatocytes (photos 2.a). In contrast, rats treated with DEN alone showed liver cuts with damaged structures and characterized by necrosis around the central vein, inflammatory cell infiltration, hot air balloon degeneration and sinusoidal dilatation (photo 2.b). However, the liver sections of the animals that received the 250 mg/kg dose extract (photo 2.d) showed a moderate degree of damage to the liver and inflammatory cells. Extracts at this dose protected the liver against hepatocyte degradation and centrilobular necrosis (photos 2.d). Histopathological examination of hepatic sections of animals treated with ethanolic extract at a dose of 250 mg/kg also showed normal hepatocytes and lacked collagen accumulation comparable to the positive control group (photos 2.e).

***a.*** *Liver (Group I)*

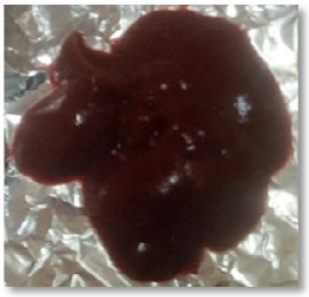


***b****. Liver (Group II)*





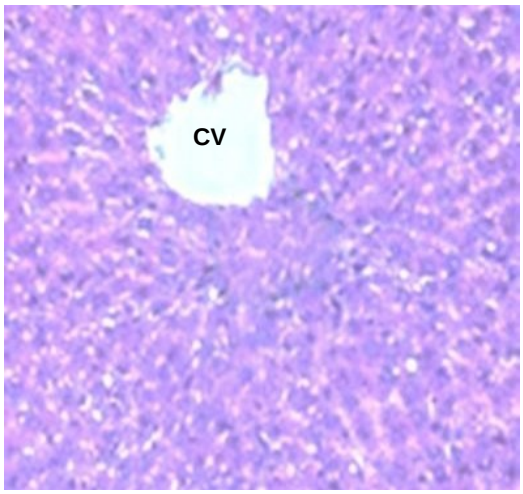
***c.****Liver (Group III)*

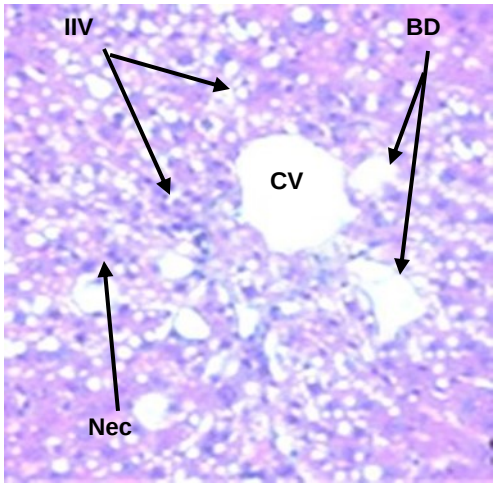


***d****. Liver (Group IV)*

***e****. Liver (Group V)*

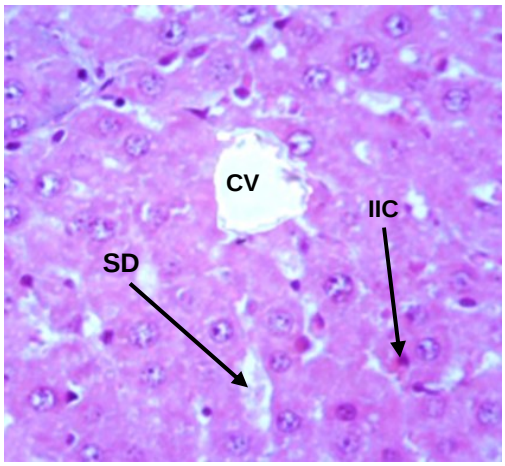
**Photo 1. Macroscopic appearance of the livers of treated animals**

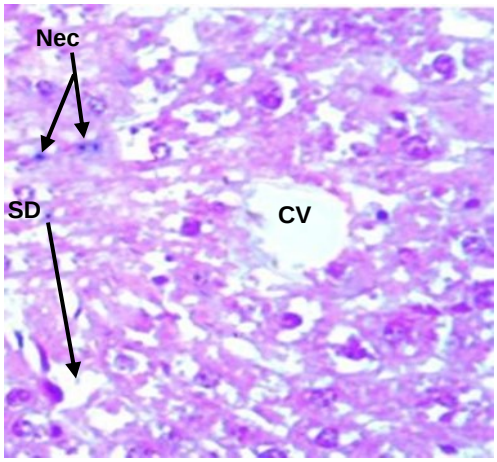



***a.*** *Group I: liver cup: Normal Group*

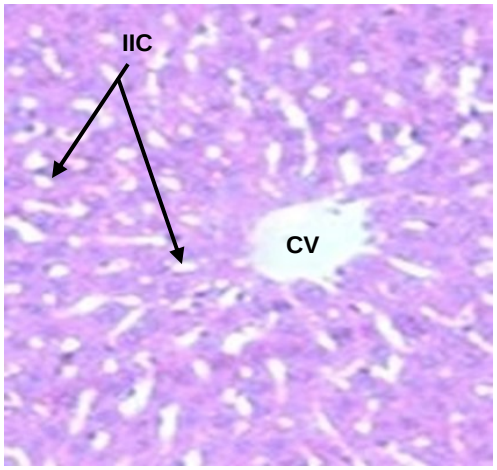
***b.*** *Group II: liver cup: negative control*




***c.*** *Group III Liver section, DEN + Silymarin.*

***d.*** *Group IV liver section, DEN + Ah (100mg/kg)*




***e.*** *Group V liver section, DEN + Ah (250mg/kg)*

**Photo 2. Structures of histopathological sections of the livers of treated rats**

*CV: central vein; IIC: Infiltration of inflammatory cells; BD: Bloating degenerations, SD: Sinusoidal dilation; Nec: Necroses (The cuts were stained with H and E, × 400)*

## 4. DISCUSSION

Fibrosis usually presents with signs and symptoms of chronic liver disease such as portal hypertension, fatigue, weight loss, hepatosplenomegaly, ascites, varicose veins and muscle atrophy [12]. Registration of the weight of animals in the negative control lot confirmed a significant loss of weight, which was improved in the test animals (100 mg/kg and 250 mg/kg body weight extract). Elevated ASAT and ALT values in animals in the negative control lot (134.67 ± 17.47 U/L and 213.67 ± 33.97 U/L) were also identified in a liver fibrosis study. human [13]. Moreover, according to Edouardo et *al*. [14], the ratio greater than 1, obtained in lot II (negative control), would show advanced liver fibrosis in these animals. The serum activity of alkaline phosphatase (ALP) comes mainly from the liver [15]. The serum PAL of the test lots showed hepatic function regulation which would prevent the establishment of fibrosis in animals receiving the dose of 250 mg/kg body weight of ethanolic extract of *Acanthospermum hispidum*. On the other hand, in animals in Lot II (negative control), high PAL values could explain a shift to cirrhosis or liver failure [16]. Compared with bilirubin and albumin values, the low levels observed in animals in lots III and V show liver synthesis capacity in these animals after the aggression [17]. The results of the histopathological analyzes were confirmed those of the histopathological studies.

In the present study, evidence of hepatotoxicity under the effect of DEN was confirmed. This hepatotoxin is likely to cause profound damage to the liver following the intensive production of free radicals causing an imbalance in the cellular redox status in favor of pro-oxidants. Indeed, it was found during the pre-test that the antioxidant defense system decreased significantly in the liver homogenates of animals of the negative control (DEN alone), leaving room for the pro-oxidants responsible for lipoperoxidation and destruction. membrane structures. The ethanolic extract of *Acanthospermum hispidum* plays a chemoprotective role against the oxidative stress produced in the cytosol and mitochondria of hepatocytes, following the administration of DEN to laboratory animals in the evaluation of the antifibrotic capacity of the extracts of *Acanthospermum hispidum*. By its ability to neutralize the reactive species produced through the metabolism of DEN [4], the ethanolic extract has shown that it has an ability to block the progression of liver fibrosis.

## 5. CONCLUSION

It is clear from this study that the ethanolic extract of *Acanthospermum hispidum* has antifibrotic properties. It is an interesting extract, rich in therapeutics, by its power to prevent the progression of liver fibrosis. The ethanolic extract at a dose of 250 mg/kg yielded interesting results in the relative weights of the animals and livers of the treated animals. Mean values for transaminase, alkaline phosphatase, total protein and total bilirubin levels observed in the animals treated with the extract were significantly improved compared to animals in the negative and positive control groups. The results of the histological studies performed on the livers of the treated animals also showed aspects of liver tissue with improved structure for group V. All of these results militate in favor of the use of the ethanolic extract of *Acanthospermum hispidum* against chronic liver infections such as fibrosis.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## CONSENT

All authors declare that "written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of

the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

## ETHICAL APPROVAL

ALL AUTHORS HEREBY DECLARE THAT "PRINCIPLES OF LABORATORY ANIMAL CARE" (ETHICAL APPROVAL CODE: 2010/63/EU, DATE OF APPROVAL: 20.10.2010) WERE FOLLOWED, AS WELL AS SPECIFIC NATIONAL LAWS WHERE APPLICABLE. ALL EXPERIMENTS HAVE BEEN EXAMINED AND APPROVED BY THE APPROPRIATE ETHICS COMMITTEE.

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