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Protective Effect of *Acanthospermum hispidum* DC (Asteraceae) Extracts against Diethylnitrosamine Induced Hepatocellular Damage

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Authors' contributions

The design of the research idea was carried out with the support of authors AT and AH. In vivo tests on hepatoprotection were conducted under the supervision of author LL. The evaluation of in vitro hepatoprotection tests was coordinated by author AH. For the benchwork, authors JYPN, DP, ENS, NO and TKT participated. Author JYPN contributed to the writing and editing of this work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Liver diseases are a major public health concern in the world. About 120 million cases of infectious HBV (the half of the world cases) are registered in sub-Saharan Africa. Burkina Faso is a country with a high endemicity of viral hepatitis. These diseases cause serious damage to the liver.
Study Design: This study aimed to assess the non-toxicity and the good hepatoprotective activities of *Acanthospermum hispidum* extracts.
Place and Duration of Study: Animal model tests and *in vitro* hepatoprotection tests were conducted from June 2017 to March 2018.
Methodology: For *in vivo* hepatoprotective potential study, rats were pretreated with *Acanthospermum hispidum* extracts at different doses and intoxicated with diethylnitrosamine. For the evaluation of the toxicity of the extracts, the OCED protocols served as a benchmark.
Results: Ethanolic extract at a dose of 250 mg/kg body weight protected the livers of animals against hepatotoxin attacks. The safety of this extract was verified at a single dose of 2000 mg/kg and repeated doses of 300 mg/kg. Approaches on the action mechanism of the extracts were assessed using the *in vitro* tests.
Conclusion: *Acanthospermum hispidum* extracts at the doses used showed hepatoprotective potential. The *in vitro* activities through the inhibition of the activity of certain enzymes involved in the physiology of hepatitis made it possible to carry out approaches on the mechanisms of action of the extracts. The proven safety combined with the hepatoprotection of the extracts justifies their use against hepatic infections.

Keywords: Acanthospermum hispidum; hepatitis; hepatoprotection; histopathology; diethylnitrosamine; Burkina Faso.

1. INTRODUCTION

Hepatitis is a serious public concern in Africa. The infectious hepatitis disease is extremely contagious. Two billion people worldwide have already been in contact with the hepatitis B virus, of which more than 350 million are chronic carriers capable of transmitting the virus for many years [1], with 800,000 cases of deaths per year. Nearly 40% of people infected with hepatitis in recent years developed hepatic complications characterised by damage to the liver such as cirrhosis, liver injury and hepatocarcinoma, the fifth most common cancer in the world [2,3]. In Burkina Faso, liver cancer is the leading cause of out-of-country health evacuation and the third leading cause of death after infectious diseases and cardiovascular diseases [4]. The pathogenicity of liver inflammation involves three enzymes, including trypsin, NADPH oxidase (NOX) and xanthine oxidase (XO). The reactive oxygenated substances produced through NOX contribute to various liver diseases, including chronic hepatitis, cholestatic liver injury and liver fibrosis [5]. Trypsin inhibitors protect liver tissues against enzymes produced by inflammatory cells, particularly elastase [6]. NADPH oxidase promotes the expression of genes encoding procollagen, fibrinogen mediators and inflammatory cytokines, which are found to be very high in the human liver in cases of hepatitis [7]. The xanthine oxidase enzyme, which has a hepatocellular origin can be released in plasma mainly during hepatocellular injury [8,9].

Hepatitis puts a heavy burden on the health care system because of the difficulties associated with the medical management of these complications, as well as the cost of conventional drugs. Faced with this limitation of therapeutic tools, the identification of new molecules especially of natural origin represents a worldwide important issue. *Acanthospermum hispidum* DC (Asteraceae) is a herb selected from an ethnobotanical survey in Burkina Faso. *Acanthospermum hispidum* is a branched plant that can reach 60 cm in height. The stems of this plant are covered with tufted hairs and small glandular hairs. This study aimed to evaluate the non-toxicity hepatoprotective activities and the mechanism of action of *Acanthospermum hispidum* bioactive molecules against diethylnitrosamine (DEN) induced hepatotoxicity in rat.

2. MATERIALS AND METHODS

2.1 Plant

The plant material consists of the whole plant of *Acanthospermum hispidum* harvested in 2017 in Loumbila. The plant was identified at the Laboratory of Plant Ecology and Botany of the

University Ouaga I Pr Joseph KI-ZERBO. Specimens were deposited at the herbarium of the Biodiversity Laboratory under identification code ID 6823. The plants were dried at room temperature away from the sun, then the dry plant material was returned in powder for extractions.

2.2 Chemical Equipment

Sigma reagents (Steinheim, Germany): Sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), EDTA (ethylenediamine tetraacetic acid), Triton X-100, GSH (glutathione reductase), NADPH, Diethylnitrosamine (DEN), p-nitroanilide N-α-benzoyl-DL-arginine, trypsin, quercetin.

Fluka chemistry reagents (Buchs, Switzerland) and prochimie: cumene hydroperoxide, DTNB, potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4), xanthine (2.6 dihydroxypurine) and xanthine oxidase, Tris-HCl. They are all analytical grade.

2.3 Extraction

2.3.1 Ethanol maceration

The whole plant powder of *Acanthospermum hispidum* was macerated (at about 30°C) in absolute ethanol at a ratio of 1:5 (mass/volume) for 24 h with mechanical stirring. The macerated whole plant powder was filtered. The filtrate that was obtained, was concentrated in an evaporator that was equipped with a vacuum pump. The extract that was obtained was frozen at minus 20°C and freeze-dried.

2.3.2 Aqueous decoction

One hundred grams (100 g) of powder is put in a flask and 500 ml of distilled water are added. The mixture is homogenised and boiled under reflux for 30 minutes. Then he is allowed to warm up. The contents of the flask are spilt in centrifuge tubes. And the supernatant is concentrated, frozen and freeze-dried.

2.4 Assessment of Toxicity

The experimental animals are Wistar variety rats and NMRI variety mice. All animals were of FSPO sanitary status (free of specific pathogenic organisms).

Upon receipt, the animals were randomly placed in groups in standard cages for an acclimation period (2 weeks) before being used in the different experiments. During this period the animals have free access to food and water (kibble from the animal feed production company) and were kept in a constant temperature (22 ± 2)°C pet shop subject to light/dark cycle of 12/12 hours. All experimental animal protocols had complied with the instructions of 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: 20.10.2010.

2.4.1 Assessment of the acute toxicity of plant extracts

The toxicity of both forms of *Acanthospermum hispidum* extract was assessed according to the recommendations of the Organization for Cooperation and Economic Development (OCED) Guidelines (OCED, No. 423) [10]. The dose of extract tolerated by the animals was determined. For this purpose, three sequential groups of three female NMRI strain mice weighing between 25 and 30 g were used and fasted four hours before the experiment. The first group is the control group and the other two groups received each a predetermined dose of 2000 mg/kg of both forms of extracts (aqueous and ethanolic). The oral route was the route of administration of the extracts through a feeding tube. The animals were observed constantly during the first thirty minutes and then regularly for the next fourteen days to note any signs of toxicity such as tremor, convulsion, salivation, diarrhoea, lethargy, sleep and coma. The same process is repeated to confirm the result obtained previously.

2.4.2 Evaluation of the sub-acute toxicity of ethanolic plant extract

The repeated-dose toxicity of *Acanthospermum hispidum* extracts was evaluated according to the guidelines of the Development Cooperation Organization [11] with some modifications. This method makes it possible to detect the signs of toxicity related to the administration of the extract at repeated doses. For this purpose, two groups of eight Wistar rats (4 females and 4 males) were used and fasted during eighteen hours before the experiment. Each rat in the test group was then dosed with a predefined daily ethanolic extract (300 mg/kg body weight), dissolved in distilled water for a period of 28 days. The animals of the control group received distilled water instead of

the extract. The oral way was the way of administration of the extract through a feeding tube. The animals were observed constantly during the first thirty minutes and then regularly to note any signs of toxicity. Food and water intake was assessed during the study. The observation was continued until the 28th day. The animals were kept fasting on the twenty-eight night and then sacrificed on a twenty-ninth day after being anesthetised. Blood from sacrificed animals was collected in two types of tubes for analysis of haematological and biochemical parameters.

The biochemical parameters were determined from the serum obtained by centrifugation of the blood collected in the non-heparinised dry tubes. Alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), blood glucose, total protein and cholesterol are measured using a kit (LABKIT) following the manufacturer's instructions. Creatinine is dosed using the SPINREACT kit according to the manufacturer's instructions.

Blood samples from heparinised tubes were used to determine hematological parameters such as hematocrit, red blood cells, hemoglobin concentration, mean hemoglobin concentration (MCHCs), mean cell volume (MCV), mean hemoglobin corpuscular rate (MCHTs), white blood cells, neutrophil granulocytes, eosinophilic granulocytes, basophilic granulocytes, lymphocytes and monocytes using the XP 300 Sysmex spectrophotometer.

The liver and kidneys were also removed and preserved in 10% formalin for histological examinations.

Recording the relative weight of the livers and kidneys of animals.

After collecting the blood samples, the organs (liver and kidneys) were carefully removed for weighing. The relative weight (Rw) of the organs was determined according to the formula:

$$Rw = Wo/Wb \times 100$$

Rw: Relative weight of organs (g/100 g); Wo: Weight of the organ (g); Wb: Rat body weight (g).

2.5 Evaluation of Hepatoprotective Activity

Diethylnitrosamine (DEN) has been used as a hepatotoxin for evaluation of the hepatoprotective activity.

2.5.1 Animal treatment

For this, Wistar rats (males) weighing between 200 and 250 g were randomised into 9 groups of six (06) rats each:

Group 1 (normal) received water by gavage.
Group 2 (negative control) received oral water and DEN (200 mg/kg) on the 7th day.
Group 3 received silymarin orally at a daily dose of 50 mg/kg body weight, a reference antioxidant.

The test groups (4, 5 and 6) receive respectively a variable dose (50, 100, 250 mg/kg body weight of ethanolic extract) and groups (7, 8 and 9) receive respectively a variable dose (50, 100, 250 mg/kg body weight of the aqueous extract) of *Acanthospermum hispidum* once a day. On the seventh day, all animals excepted rats of group 1 received DEN (200 mg/kg body weight) intraperitoneally. On the 8th day, the rats of the nine groups were sacrificed.

2.5.2 Histopathological analyses

The livers of the treated animals were collected, weighed and used for histological analysis in the cytogenetics laboratory. The methodology described by Hould [12] was used. Liver sections (about 0.2 × 0.2 cm) were made with the rotating microtome. These sections were fixed with 10% formalin and then placed in a paraffin bath. The liver slices were then labelled with hematoxylin-eosin. Finally, the marked liver slices were subjected to microscopic examination for histological analysis.

2.6 *In vitro* Hepatoprotective Activities

2.6.1 Inhibition of NADPH oxidase involving glutathione peroxidase assay (GSPX)

The inhibitory activity of *Acanthospermum hispidum* extracts was evaluated by the method described by Nagalakshmi and Prasad [13] with some modifications. The reaction mixture consisted of 10% liver homogenate in Tris-HCl (100 mM, pH 7.5), 1 mM EDTA, 0.2% (w/v) Triton X-100, 1 mM GSH. 0.15 mM NADPH and 1 mM cumene hydroperoxide for the purpose of releasing NADPH oxidase. 50 µL of extract (100 µg/mL) and 50 µL of DTNB (2 mM) are added to 150 µL of the reaction mixture. Incubation was done at 30°C for 10 minutes. Under these conditions, GSH reacts with the substrate to produce 2-nitro-5-thiobenzoic acid as well as glutathione-disulfide (GSSG) under the action of

glutathione peroxidase. The rate of oxidation of NADPH was measured by monitoring the formation of 2-nitro-5-thiobenzoic acid, a yellow colouring product that absorbs at 412 nm. Quercetin and gallic acid were used as a reference. The absorbance variation (ΔDO) measured between one and three minutes (ΔT) made it possible to determine the percentage inhibition of NADPH oxidase according to the formula: I% = (Vc-Vo)/Vc × 100.

V_C = Variation of the absorbance per minute of the test without extract.
V_0 = Variation of the absorbance per minute of the test with extract.

2.6.2 Inhibition of trypsin assay

The inhibitory capacity of trypsin by extracts was evaluated according to the method described by Arefrad et al. [14]. To measure the inhibitory activity of the extracts on trypsin, 5 µg of trypsin and 5 µg of protein extract (100 µg/mL) were incubated for five (5) minutes in a volume of 800 µL of buffer (0.2 M Tris-HCl pH 7 8). 160 µg of p-nitroanilide N-α-benzoyl-DL-arginine (BAPNA) dissolved in 200 µL of buffer (0.05 M Tris-HCl pH 8.2, 0.05 M CaCl2) were added to the reaction mixture. The reading was made for 25 minutes at 410 nm using a spectrometer. The percent inhibition (I%) was calculated from the following equation:

I% = (E-S)/E × 100

Where E is the activity of the enzyme without inhibitor and S its activity in the presence of the extract to be tested.

2.6.3 Inhibition of xanthine oxidase assay

Inhibition of xanthine oxidase activity was achieved by the spectrophotometric method as described by Owen and Johns [15] with some modifications. The test consisted in mixing 150 µL of phosphate buffer (0.066 M, pH 7.5), 50 µL of a test extract solution (100 µg/mL) and 50 µL of the enzyme solution (0.28 µL/mL). After 3 minutes of incubation at a temperature of 25°C, the reaction was initiated by the addition of 250 µL of phosphate buffer solution (0.066 M, pH 7.5) of xanthine (0.15 M). A control consisting of a xanthine oxidase solution was also prepared. The reaction was initiated for 3 minutes at the wavelength of 295 nm and the reaction velocity V0 was measured. The phosphate buffer was used as a negative control (the activity of the enzyme without the extract solution), and allopurinol as a positive control. The percentage inhibition of xanthine oxidase (I%) was determined according to the equation described below:

I% = (Vc-V0)/Vc × 100

VC = variation of the absorbance per minute of the test without extract, V0 = variation of the absorbance per minute of the test with an extract

2.7 Statistical Analysis

The graphical representation of the data was performed using the Graph Pad Prism 5.0 software (Microsoft, USA). The mean value is accompanied by the standard error on the mean (mean ± standard deviation). The difference between the two values is significant when P=0.05.

3. RESULTS

3.1 Description of the Experimental Results

3.1.1 Evaluation of toxicity

3.1.1.1 Acute toxicity of extracts

The OCED method allowed the evaluation of extracts in the Globally Harmonised Classification System (GHS) of substances that could lead to acute toxicity. The method is based on a sequential process using a few animals and aimed at obtaining sufficient information for classification purposes. The acute toxicity of ethanolic and aqueous extracts of *Acanthospermum hispidum* was evaluated. At the dose of 2000 mg/kg body weight, no deaths were observed among the NMRI mice for both extracts (Table 1). This result leads to the conclusion that *Acanthospermum hispidum* extracts are not toxic. Thus the LD 50% was estimated at 5000 mg/kg of body weight. This result allowed to select the doses of extract (50, 100 and 250 mg/kg of body weight) to be administered to the animals for the continuation of our tests *in vivo*.

3.1.1.2 Subacute toxicity

Repeated dose toxicity was assessed through analysis of haematological parameters, biochemical parameters, animal weight records, relative organ weight (liver and kidney), and

Table 1. Animal observation results after oral administration of the aqueous and ethanolic extract of *Acanthospermum hispidum*

Clinical signs of toxicity by a batch of animals	Lot 1 (normal)	Lot 2 (Aqueous extract) 2000 mg/kg	Lot 3 (Ethanolic extract) 2000 mg/kg
Breathing disorders	-	-	-
Refusal of food	-	-	-
Oral bleeding	-	-	-
Nasal bleeding	-	-	-
Abdominal pain (contortion)	-	-	-
Coma	-	-	-
Diarrhoea	-	-	-
Mortality	-	-	-

Absent: -

Table 2. Results of haematological parameters

Parameters	Control	Test (ethanolic extract)
Hatiaties (10^6)	7.54±1.45	8.45±1.5
Hematocrit (%)	45.95±2.13	45.7±2.10
Hemoglobin (g/dl)	15.01±1.54	13.8±1.02*
VGM (fl)	63.14±2.01	60.6±2.1
MCHTs (pg)	20.48±2.31	18.3±1.52
$MCHC_s$ (%)	32.45±3.5	30.2±4.12
Leukocytes $(mm^3)^{-1}$	6200±2	6400±1
Neutrophil $(mm^3)^{-1}$	4700±7.4	4620±5.2
Lymphocyte $(mm^3)^{-1}$	3890±4.46	3780±7.94
Platelets ($10^3/mm^3$)	270±3.48	273±3.41

Group 1: control; Group 2: ethanolic extract. Mean ± SD (n = 8). Anova 1 way, t-test, P=0.05 the difference is not significant; Compared to control

organ histology (liver and kidney). From the analysis of the haematological parameters, it appears that there was no significant difference between the haematological parameters of the animals which received the ethanolic extract at the dose of 300 mg/kg and those of the control for the red blood cells. hematocrit, MCHTs, MCHCs, leukocytes, neutrophils, and lymphocytes (Table 2).

The biochemical parameters of the control and the test showed that there was no significant difference between the control group and the test group (ethanolic extracts 300 mg/kg) at the level of the blood sugar, the proteinemia, the serum creatinine, the cholesterol, the ASAT and ALAT (p= 0.05) (Table 3).

The Variations in animal weight, the relative weight of organs (liver and kidney) between control and test were recorded in Tables (Table 4 and Fig. 1). The results showed that there is not a significant difference between the animal weight and the relative weight of the organs in the control group and the test group.

The observation of histopathological sections of the organs (kidneys and liver) of the animals treated with the ethanolic extract showed a normal architecture (Fig. 2). These observations confirm that the ethanolic extract is not toxic at a dose of 300 mg/kg.

Table 3. Results of biochemical parameters

Parameters	Control	Test (ethanolic extract)
Blood glucose (g/L)	1.06±0,05	1.08±0,15
Proteinemia (mg/L)	65±1.25	64±2.35
Creatinine (mg/L)	7±2	7.02±1.4
Cholesterol (g/L)	0.54±0.01	0.53±0.00
ALAT (U/L)	90.58±5.75	86.8±5.23
ASAT (U/L)	201±8.41	198±5.46

Group 1: control; Group 2: ethanolic extract. Mean ± SD (n = 8). Anova 1 way, t-test, P=0.05 the difference is not significant; Compared to control

3.1.2 *In vivo* hepatoprotective activity results

The results of the microscopic observations showed a significant difference between the liver architecture of the animals in the control group

and that of the liver of the animals in the negative control group (Fig. 3.a) and (Fig 3.b). At the level of liver sections in animals that received ethanolic extract at 250 mg/kg body weight, the tissues showed a regular appearance with small infiltrations (Fig. 3.f). In contrast, the liver sections of animals treated with DEN alone show necrosis and focal pycnosis (Fig. 3.b). The liver cells of group 2 animals (negative control) showed a lot of necrosis with degeneration. Thus, the regular appearance of liver tissue in animals pretreated with ethanolic extract demonstrates the ability of this extract at a dose of 250 mg/kg of body weight to protect the liver against the aggression of DEN (hepatotoxin).

Table 4. Results of variation in animal weight

Groups	1st day	1st week	2nd week	3rd week	4th week
Control	224±10	237±7	249.33±13.65	254±11.13	265±14.14
Test	212±8.88	223±7.45	233.67±12.86	245.33±4,16	261±18.38

Group 1: control; Group 2: ethanolic extract. Mean ± SD (n = 8)

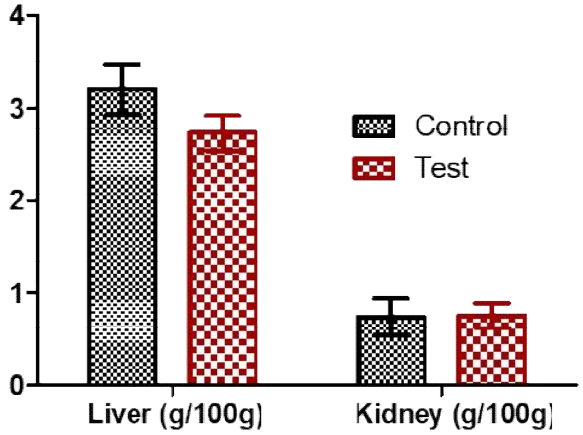
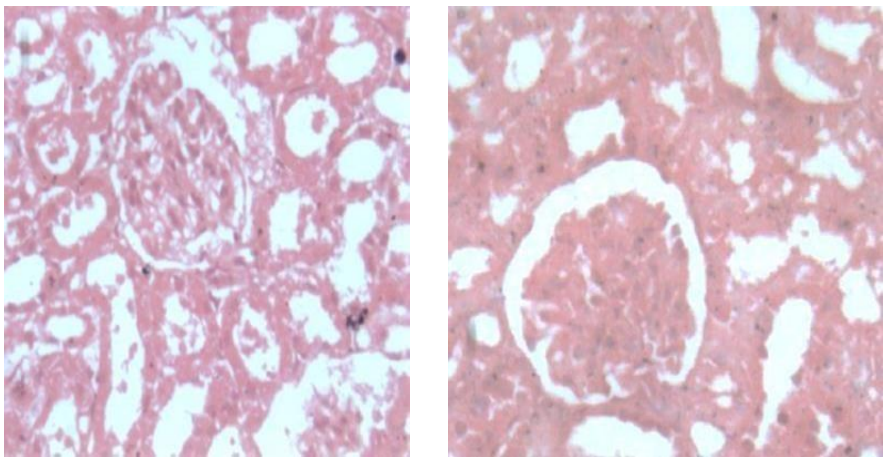
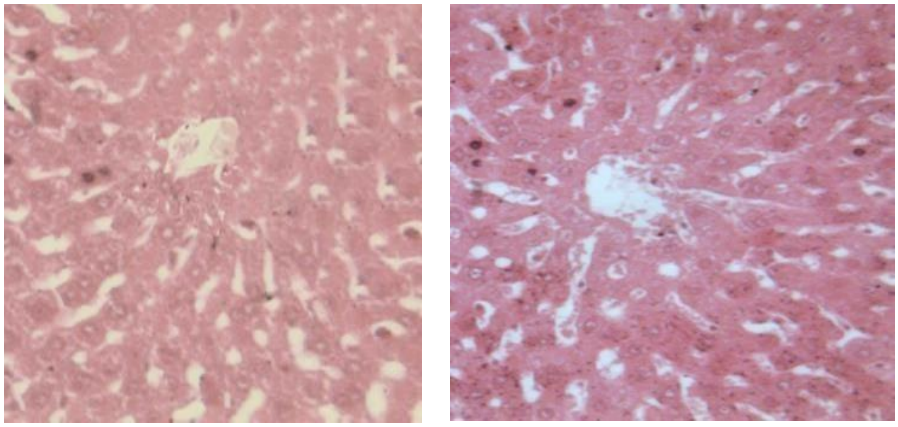


Fig. 1. Results of the relative weight of organs

Group 1: Control; Group 2: Test (ethanolic extract). Mean ± SD (n = 8). Anova 1 way, t-test: P=0.05 the difference is not significant; Compared to the control



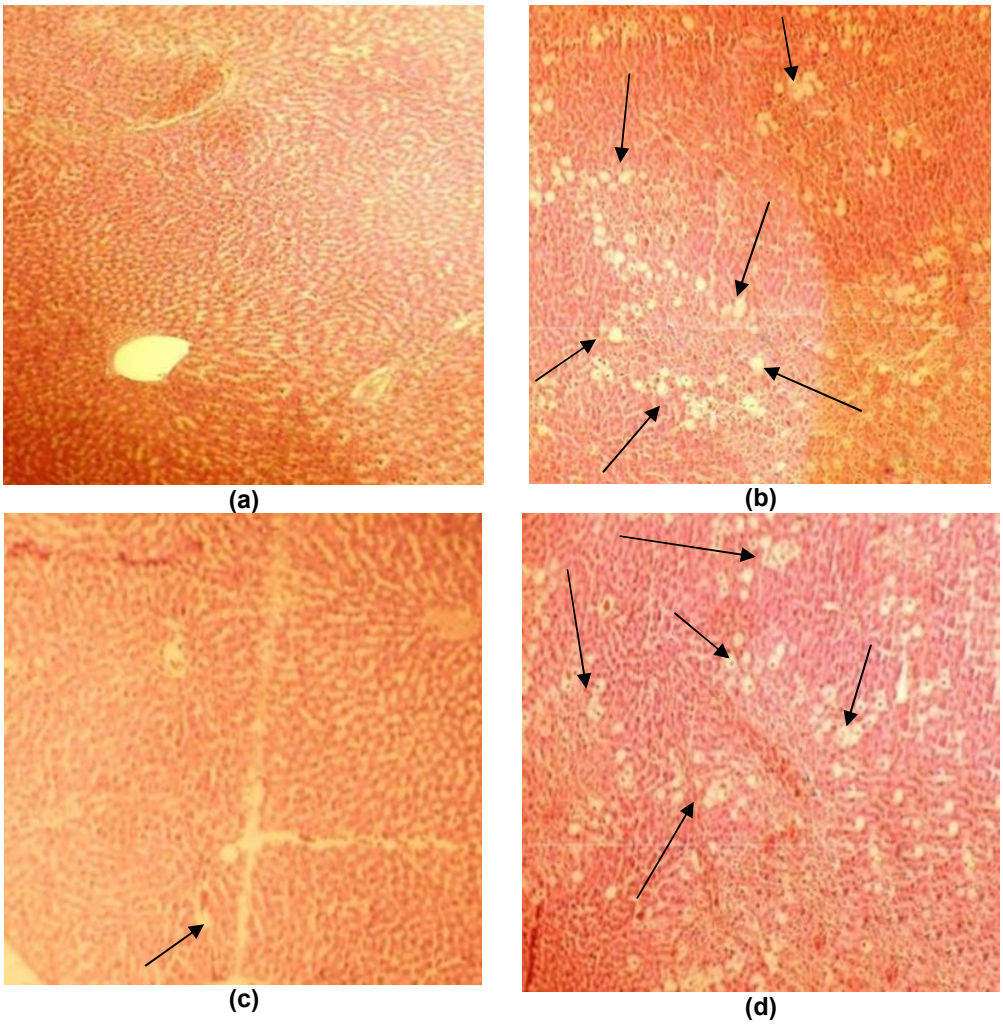
(a) **(b)**



(c) (d)

Fig. 2. Histological sections of livers and kidneys

(a) Kidney (Control); (b) Kidney (Test: Ethanolic Extract); (c) Liver (Control); (d) Liver (Test: Ethanol Extract). The cuts were stained with H and E, ×100



(a) (b)

(c) (d)

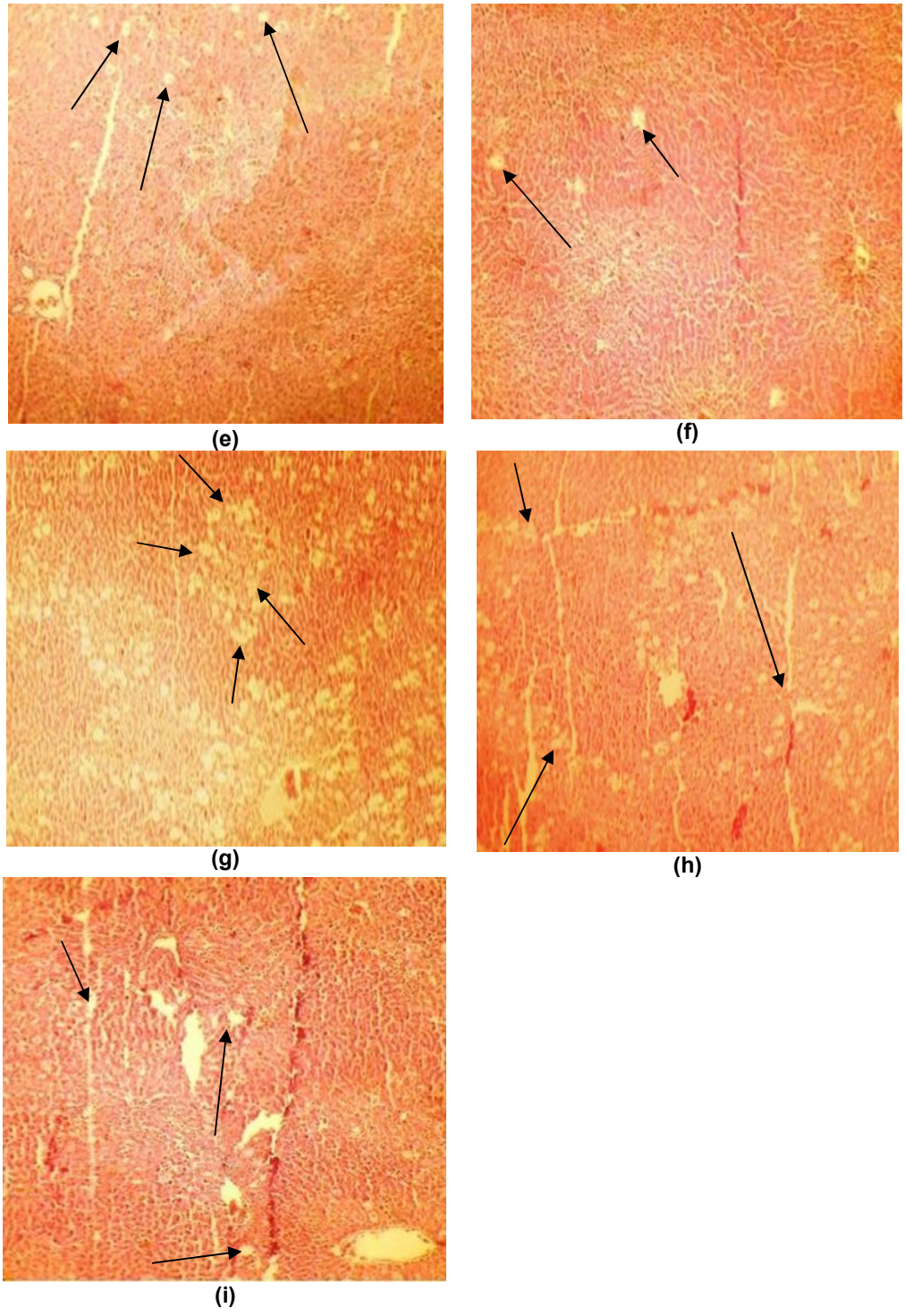


Fig. 3. Histological sections of livers

(a) Liver (Group 1: control); (b) Liver (Group 2: negative control DEN); (c) Liver (group 3: silymarin 50 mg/kg); (d) Liver (group 4: Ethanol Extract 50 mg/kg); (e) Liver (group 5: Ethanol Extract 100 mg/kg); (f) Liver (group 6: Ethanol Extract 250 mg/kg); (g) Liver (group 7: Aqueous Extract 50 mg/kg); (h) Liver (group 8: Aqueous Extract 100 mg/kg); (i) Liver (group 9: Aqueous Extract 250 mg/kg); The cuts were stained with H and E, ×10

3.1.3 Results of *in vitro* hepatoprotective activities

3.1.3.1 Results of inhibition of NADPH oxidase

Evaluation of the percent inhibition of NADPH oxidase involving glutathione peroxidase had shown that the ethanolic extract of *Acanthospermum hispidum* was more active than the aqueous extract. In addition, compared to quercetin (reference compound), the ethanolic extract (81.03 ± 5.80%) had a better percent inhibition (Table 5).

3.1.3.2 Results of Inhibition of trypsin

The aqueous extract showed higher inhibition percentage on trypsin enzyme than the ethanolic extract of *Acanthospermum hispidum*. Gallic acid used as reference compound gave a percentage inhibition of 76.31 ± 3.59 at an initial concentration of 100 µg/mL (Table 5).

3.1.3.3 Results of inhibition of xanthine oxidase

From the results of xanthine oxidase inhibition of the extracts, the ethanolic extract exhibited a high percentage inhibition compared to the aqueous extract of *Acanthospermum hispidum*. Compared with allopurinol (reference inhibitor), the percentage inhibition of the ethanolic extract (35.05 ± 2.57) is relatively low at a dose of 100 µg/mL (Table 5)

4. DISCUSSION

NADPH oxidase is an enzyme involved in the pathogenesis of the liver. This enzyme produces reactive oxygen species (ROS) secondary to their damage, which may be proteolysis but are often caused by oxidative stress itself [16]. The ethanolic extract that had the best inhibitory capacity on NADPH oxidase compared to reference compounds could be used to protect the liver against factors promoting inflammation of the liver [17]. Hepatocytes are usually damaged by the production and accumulation of trypsin [18]. This results in necrosis, inflammatory reaction and fibrosis, the main complications of which are: hyperammonemia (the detoxifying effect of the liver being impaired), jaundice or hepatocarcinoma [17]. Thus, the inhibitory activity of *Acanthospermum hispidum* extracts on trypsin could demonstrate a capacity of extracts to block the evolution of liver fibrosis. In addition, this anti-inflammatory activity is corroborated by the results of the inhibitory activity on xanthine oxidase. Thus ethanolic extract could prevent the accumulation of procollagen, fibrinogen mediators and inflammatory cytokines [19]. The anti-inflammatory activity is supported by the hepatoprotective activity of the ethanolic extract of *Acanthospermum hispidum*, which at a dose of 250 mg/kg was able to protect the liver of rats treated against the aggressions of DEN [20]. The results of a previous study had shown through the analysis of biochemical parameters and *in vivo* antioxidant activities that our extracts possessed a hepatoprotective capacity [21] and which were confirmed by this histological study of liver sections. Verification of the safety of extracts of the species is necessary as long as these extracts have been active in investigations of *in vitro* anti-inflammatory activity and hepatoprotective activity *in vivo*. Acute toxicity results for both forms of extracts did not show signs of toxicity at the end of the study. These results obtained at a dose of 2000 mg/kg body weight show that the extracts can be classified in category 5 (not classified) according to the OCED guideline 423 [10] and considered as non-toxic substances [22]. These results are corroborated in another study in which it was found that the ethanolic extract of *Acanthospermum hispidum* was not toxic at a dose of 2000 mg/kg body weight [23]. In addition, the sub-acute oral toxicity study of the ethanolic extract of *Acanthospermum hispidum* at a dose of 300 mg/kg body weight revealed that throughout the study period it did not observe mortality or morbidity within the batch of

Table 5. Results of inhibition percentages of extracts on xanthine oxidase, trypsin and NADPH oxidase

Extract/ reference compounds (100 µg/mL)	Inhibition of xanthine oxidase (%)	Inhibition of trypsin (%)	Inhibition of NADPH oxidase (%)
Ethanolic extract	35.05±2.57^{c}	17.22±4.74^{c}	81.03±=2.80^{a}
Aqueous extract	15.15±1.23^{d}	48.09±1.69^{b}	20.60±0.60^{d}
Quercetin	-	-	76.85±2.51^{b}
Gallic acid	55.89±1.29^{b}	76.31±3.59^{a}	68.98±3.25^{c}
Allopurinol	95.86±0.54^{a}	-	-

The results presented in the columns of the table with the letters (a-d) are significantly different at P=0.05

animals treated with the extract. Similarly, no treatment-related changes in body weight and organ weight of treated animals were observed. The relative body weight gain of the animals treated with the extract would be a simple index that could show improvement in the nutritional status of the animals [24]. Haematological parameters were also evaluated for further information related to toxicity. These studies on haematological parameters did not reveal abnormalities in metabolic processes of the body in the animals treated with the extract [25]. In addition, histological examination of internal organs (kidneys and liver), from treated animals and controls had shown the normal architecture, suggesting no adverse changes and morphological disturbances after 28 days. The hematopoietic system is highly sensitive to toxic compounds and serves as an important indicator of the physiological and pathological state of animals and humans [26]. In this study, haematology showed no significant change in the parameters of treated rats compared to controls. However, a slight variation was observed for some parameters such as haemoglobin, platelets and MCV. The relatively increased number of leukocytes would be useful in stimulating the immune system [27]. These results may explain the hepatoprotective activity of *Acanthospermum hispidum* DC (Asteraceae) extracts, which could be good stimulants of the immune system in chronic hepatitis cases [28]. Similarly, the non-significant differences observed in the biochemical parameters of the animals treated with the extract, such as serum creatinine and transaminases (ALAT and ASAT), are effective indicators of good renal (functional nephron) and hepatic function (hepatocyte function) in these animals [20]. This finding was confirmed by the analysis of histological sections made with the livers and kidneys of the rats.

5. CONCLUSION

The ethanolic extract of *Acanthospermum hispidum* plays a chemoprotective role against the oxidative stress produced in the cytosol of hepatocytes during the administration of DEN to laboratory animals. By its ability to neutralise the reactive species produced by the metabolism of DEN, the ethanolic extract has shown that it has an anti-hepatotoxic capacity. The results of the histopathological studies confirmed the hepatoprotective potential of this extract at the dose of 250 mg/kg of body weight. In addition, the results of *in vitro* bioassays (inhibition of NADPH oxidase, trypsin and xanthine oxidase) demonstrated *in vitro* hepatoprotective ability of extracts. It is clear from this study that the ethanol extract whose safety has been proven by the toxicity assessment (acute and subacute) is an interesting extract, rich in therapeutic agents.

CONSENT

It is not applicable.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. Global Vaccine Safety Initiative (GVSI) Fourth Meeting; 2015. (French)
2. Sartor C. Thèse de Doctorat, Docteur de l'Université Paris-Sud Ecole, France; 2016. (French)
3. Zhu RX, Seto WK, Lai CL, Yuen MF. Epidemiology of hepatocellular carcinoma in the Asia-Pacific region. Gut Liver. 2016;10:332–339.
   DOI: https://doi.org/10.5009/gnl15257
4. Diarra B, Ouattara AK, Djigma WF, Compaore TR, Obiri-Yeboah D, Traore L, Soubeiga ST, Bado P, Yara J, Pietra V, Ouedraogo P, Bougouma A, Sanogo R, Simpore J. World hepatitis day in Burkina Faso, 2016: Awareness, screening, identification of HBV markers, HBV/HCV coinfection, and vaccination. Hepat. Mon. 2017;17:1-8.
   DOI: 10.5812/hepatmon.13789
5. Li S, Li S, Tan H, Wang N, Zhang Z, Lao L, Wong C, Feng Y. The role of oxidative stress and antioxidants in liver diseases. Int. J. Mol. Sci. 2016;16:26087–26124.
   DOI: 10.3390/ijms161125942
6. Korkmaz B, Horwitz M, Jenne D, Gauthier F. Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in

human diseases. Pharmacol. Rev. 2010;62:726–759.
DOI: 10.1124/pr.110.002733.726

7. Liang S, Kisseleva T, Brenner DA. The role of NADPH oxidases (NOXs) in liver fibrosis and the activation of myofibroblasts. Front. Physiol. 2016;7:1–10.
DOI: 10.3389/fphys.2016.00017
8. DO MB, Vuppalanchi R, Chalasani N. Febuxostat-induced acute liver injury. Front. Physiol. 2017;63:1047–1049.
DOI: 10.3389/fphys.2016.00017
9. Battelli MG, Bolognesi A, Polito L. Biochimica et Biophysica Acta Pathophysiology of circulating xanthine oxidoreductase : New emerging roles for a multi-tasking enzyme. BBA-Mol. Basis Dis. 2014;1842:1502–1517.
DOI:https://doi.org/10.1016/j.bbadis.2014.05
10. OCDE. Ligne directrice de l'OCDE pour les essais de produits chimiques toxicité orale aiguë - méthode par classe de toxicité aiguë. 2001;427:1-14. (French)
11. OCDE. Ligne directrice de l'OCDE pour les essais de produits chimiques 407; 2008b. (French)
12. Hould R. Techniques d'histopathologie et de cytopathologie. Maloine; 1984. (French)
13. Nagalakshmi N, Prasad MNV. Responses of glutathione cycle enzymes and glutathione metabolism to copper stress in *Scenedesmus bijugatus*. Plant Sci. 2001;160:291–299.
14. Arefrad M, Jelodar NB, Nematzadeh G, Kazemitabar S. Influence of genotype variation on trypsin and chymotrypsin inhibitors levels of seed storage proteins composition in soybean [*Glycine max* (L.) Merrill]. Int. J. Agron. Plant Prod. 2013;4:2877–2884. ISSN: 2051-1914.
15. Owen PL, Johns T. Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. J. Ethnopharmacol. 1999;64:149–160.
16. Kennedy AR. The Bowman-Birk inhibitor from soybeans as an anticarcinogenic agent the Bowman-Birk inhibitor from soybeans as an. Am. J. Clin. Nutr. 2017;10:1–8.
17. Nusrat S, Khan MS, Fazili J, Madhoun MF. Cirrhosis and its complications: Evidence based treatment. World J. Gastroenterol. 2014;20:5442–5460.
DOI: 10.3748/wjg.v20.i18.5442
18. Reilly LPO, Perlmutter DH, Silverman GA, Pak SC. α1-antitrypsin deficiency and the hepatocytes- an elegans solution to drug discovery. Int J Biochem Cell Biol. 2015;47:109–112.
19. Boudreau HE, Emerson SU, Korzeniowska A, Jendrysik MA, Leto TL. Hepatitis C virus (HCV) proteins induce NADPH oxidase 4 expression in a transforming growth factor-dependent manner : A new contributor to HCV-induced oxidative stress. J. Virol. 2009;83:12934–12946.
DOI: 10.1128/JVI.01059-09
20. Debelo N, Afework M, Debella A, Makonnen E. Histopathological and biochemical assessment of chronic oral administration of aqueous leaf extract of *Thymus serrulatus* in mice. J. Clin. Exp. Pathol. 2015;05:1-9.
DOI: 10.4172/2161-0681.1000258
21. N'do JY, Hilou A, Ouedraogo N, Sombié EN, Traoré TK. Phytochemistry, antioxidant, and hepatoprotective potential of *Acanthospermum hispidum* DC extracts against diethylnitrosamine-induced hepato-toxicity in rats. Medicines. 2018;42:1–13.
DOI: 10.3390/medicines5020042
22. Azeez L, Salau AK, Adewuyi SO, Osineye SO, Tijani KO, Balogun RO. Safety evaluation of Osun River water containing heavy metals and Volatile Organic Compounds (VOCs) in rats. Niger. J. Physiol. Sci. 2015;30:103–109.
23. Himaja N. Comparitive study of hepato-protective activity of *Acanthospermum hispidum* plant extract and herbal niosomal suspension against anti-tubercular drug induced hepatotoxicity in rats. Asian J. Pharm. Clin. Res. 2015;8:301–304. ISSN: 0974-2441.
24. Awodele O, Adeneye AA, Aiyeola SA, Benebo AS. Modulatory effect of *Mangifera indica* against carbon tetrachloride induced kidney damage in rats. Interdiscip. Toxicol. 2015;8:175–183.
DOI: 10.1515/intox-2015-0027
25. Andreoli V, Sprovieri F. Genetic aspects of susceptibility to mercury toxicity: An overview. Int. J. Environ. Res. Public Health. 2017;14:53-93.
DOI: 10.3390/ijerph14010093
26. Asiimwe S, Karlsson AB, Azeem M, Mugisha KM, Namutebi A, Gakunga NJ. Chemical composition and toxicological evaluation of the aqueous leaf extracts of *Plectranthus amboinicus* Lour. Spreng. Int. J. Pharm. Sci. Invent. 2014;3:19–27.
27. Morsy GM, El-Ala KSA, Ali AA. Studies on fate and toxicity of nanoalumina in male

albino rats: Some haematological, biochemicaland histological aspects. Toxicol. Ind. Health. 2013;32:634–655. DOI: 10.1177/0748233713504022

28. Palozi RAC, Schaedler MI, Tirloni CAS, Silva AO, Lívero FADR, Souza RIC, Dos Santos AC, Prando TBL, De Souza LM, Gasparotto A. Roles of nitric oxide and prostaglandins in the sustained anti-hypertensive effects of *Acanthospermum hispidum* DC. on ovariectomized rats with renovascular hypertension. Evidence-based Complement. Altern. Med. 2017;14. DOI: https://doi.org/10.1155/2017/2492483

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