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Research Paper

IN VIVO HEPATOPROTECTIVE POTENTIAL AND ANTIOXIDANT PROPERTIES OF FRACTIONS DERIVED FROM THE ETHANOLIC EXTRACT OF Acanthospermum hispidum DC

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Abstract

Acanthospermum hispidum is an herb of the Asteraceae family. This plant has been registered as a medicinal plant used in Burkina Faso against liver diseases. Previous results have confirmed the hepatoprotective potential of the ethanolic extract of this plant. Phytochemical, antioxidant and anti-hepatotoxic tests were used to evaluate the hepatoprotective potential of the fractions derived from the ethanolic extract of *Acanthospermum hispidum*. Assay test results showed total phenol and total flavonoid levels in the fractions. The fraction that exhibited the best in vivo hepatoprotective activity through the values of *in vivo* antioxidant enzymes such as catalase (0.19 U / 10 mg) and superoxide dismutase (0.338 U / mg), and transaminases characteristic of hepatocytes such as ASAT and ALAT also showed the best levels of phenolic compounds. Phenolic compounds in general and flavonoids specifically are recognized to possess hepatoprotective properties. These high levels of total flavonoids of the ethyl acetate and dichloromethane fractions with respectively 41.21±4.99 and 47.76±5.09 mg EQ/g extract, could justify the hepatoprotective potential of the ethyl acetate and dichloromethane fractions. The modes of action of the fractions to protect the liver were evaluated through them *in vitro* antioxidant capacity and them *in vitro* biomembrane protective capacity. These results promise the enrichment of *Acanthospermum hispidum* extracts in hepatoprotective molecules against liver diseases.

Key words: *Acanthospermum hispidum, Phenolic compounds, antioxidant, protection of biomembranes, hepatoprotection in vivo*.

INTRODUCTION

The liver is the target of various attacks (viruses, alcohol, chemical substances present in the diet or the living environment) [1]. It also undergoes profound pathological changes, such as diabetes or obesity [2]. Liver involvement is generally characterized by an increase in certain biochemical parameters such as transaminases and a decrease in antioxidant enzymes [3]. The hepatic parenchyma can react to these aggressions in an acute way resulting in fulminant hepatitis where the massive necrosis of the hepatocytes causes a hepatic insufficiency which can be lethal. However, a chronic response or chronic hepatitis often occurs, characterized by the development of varying degrees of hepatocyte necrosis, tissue infiltration of inflammatory cells, and fibrosis. The progression of these pathological conditions can lead to the development of hepatic cirrhosis or even liver cancer [4]. 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 alternatives, the identification of new molecules especially of natural origin represents an important issue.

Acanthospermum hispidum DC (Asteraceae) is an herb selected from an ethnobotanical survey in 2014 that identified the medicinal plants used in the management of liver diseases by traditional medicine in Burkina Faso [5]. The present work consisted in evaluating the hepatoprotective potential and performing a phytochemical investigation with the in *vitro* antioxidant capacity of the fractions obtained from the ethanolic extract which had a better hepatoprotective activity compared to the aqueous form [6].

1. MATERIALS AND METHODS

1.1. Plant

Acanthospermum hispidum was harvested in 2018 in Loumbila. This plant has been identified and authenticated by the laboratory of plant ecology and botany of University Ouaga I Pr Joseph KI-ZERBO. Specimens were deposited at the herbarium of the Biodiversity Laboratory under identification code ID 16875. The harvested plants were dried at room temperature away from the sun, then the dry matter was reduced to powder.

1.2. Chemical equipment

Sigma reagents (Steinheim, Germany): Sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), EDTA (ethylenediamine tetraacetic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzolin-6-sulphonate) (ABTS), 2-2-Deoxy D-ribose, Hydrogen Peroxide, Nitro Chloride Blue Tetrazolium, Silymarin, Diethylnitrosamine (DEN), Ascorbic Acid, Gallic Acid, Quercetin, Trolox.

Fluka chemie reagents (Buchs, Switzerland) and prochimie: potassium hexacyanoferrate [$K_3Fe(CN)_6$], iron chloride [$FeCl_3$], iron sulphate, trichloroacetic acid and thiobarbituric acid. They are all analytical grade.

1.3. Extraction

The whole plant powder of *Acanthospermum hispidum* was macerated (about 30 °C) in absolute ethanol for 24 hours with mechanical stirring. The macerated was filtered. The filtrate obtained in brown-green, oily in appearance was concentrated in an evaporator equipped with a vacuum pump and then evaporated to dryness. The crude extract obtained was frozen at minus 20 °C and freeze-dried for subsequent investigations.

This extract was fractionated with the following solvents: n-hexane, water, dichloromethane, ethyl acetate, and methanol according to the method described by Lompo et *al*. [7] with some modifications.

1.4. Phytochemical investigation

Determination of total polyphenols

The determination of total phenolic was carried out according to the method of Singleton et *al.* [8] with some modifications. This method is based on colorimetry, using Folin ciocalteu. One hundred and twenty-five microliters of Folin reagent (0.2 mol/L) was added to 25 µL of extract. The reaction mixture was then allowed to incubate for 2 hours at room temperature. A spectrophotometer reading, using a microplate reader, was taken at 760 nm. Gallic acid was used as a reference to plot the standard curve ($y = 201x - 21.22$, $r^2 = 0.99$) and the result was expressed in milligram Equivalent Gallic acid per gram of extract (mg GAE/g).

Determination of total flavonoids

The flavonoid assay was performed according to the method described by Compaoré et *al*. [9]. One hundred microliters of AlCl3 (2%) was added to the extract (1 mg/mL). The mixture was made in the microplate wells and then allowed to incubate for 15 minutes. The reading was taken at 415 nm against a quercetin standard curve (y = 39.8x − 3.5, r2 = 0.99) and the amounts were expressed in milligram Equivalent Quercetin per gram of extract (mg QE/g).

1.5. Determination of the *in vitro* antioxidant potential of extracts of *Acanthospermum hispidum* DC.

In order to evaluate *in vitro* the antioxidant potential of *Acanthospermum hispidum* extracts, antioxidant models (DPPH, ABTS, FRAP) were used. Several factors intervene in the measurement of the antioxidant capacity of a matrix namely, the chemical reactivity of the antioxidant with respect to the radical that is to be trapped, the destination of the radical that is derived from the antioxidant, the interaction with other antioxidants, concentration and surrounding mobility, absorption, distribution, retention, and metabolism of the antioxidant.

Inhibition of Radical ABTS Assay

This method was based on the decolorization of the stable radical cation $ABTS^{\bullet+}$ [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)]. The disappearance of the blue-green chromophoric radical $ABTS^{\bullet+}$ was monitored at 734 nm, according to the method described by Re et *al*. [10]. The sample that was to be tested (500 µg/mL in 50 µL of methanol) was incubated for 15 minutes in the dark with 200 µL of freshly prepared $ABTS^{\bullet+}$ solution. The absorbance at 734 nm was measured, using a spectrophotometer, against a standard trolox curve ($y = -72.38x + 54.57$, $r^2 < 0.99$, $p < 0.001$). The experiment was carried out in triplicate (independent tests) and the anti-radical activity of the extract, by reduction of the radical cation $ABTS^{\bullet+}$, was expressed in millimoles Equivalent Trolox per gram of extract (mmol TE/g).

Reduction of Iron III Assay (FRAP)

The reducing power of the extracts method was based on the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) by the reducing compounds, following a mono-electron

transfer. The determination of the reducing power of *Acanthospermum hispidum* extracts was evaluated as described by Hinneburg et *al*. [11]**,** with some modifications. In a test tube, 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of potassium hexacyanoferrate (1% aqueous) were added to 0.5 mL of test extract (0.6 mg/mL). The mixture was heated at 50 °C in a water bath for 30 minutes. After cooling, trichloroacetic acid (1.25 mL, 10%) was added and then the mixture was centrifuged (2000× *g* for 10 minutes). Three aliquots (125 µL) of the supernatant were transferred into a 96-well microplate, after which 125 µL of distilled water and then 25 µL of $FeCl_3$ (0.1% aqueous) were added. The reductive power was evaluated at 700 nm, against a standard curve of ascorbic acid (y = 105.9x, $r^2 > 0.99$, $p < 0.0001$). The experiment was carried out in triplicate (independent tests) and the reducing activity of the extract was expressed in milligram Equivalent Ascorbic Acid per gram of extract (mg AAE/g).

Inhibition of radical DPPH assay

The anti-radical activity of the crude extracts was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, as described by Alisi et *al*. [12]. This method is based on the reduction of the absorbance of the stable free radical DPPH• at 517 nm, in the presence of an H• radical donor. A series of seven successive dilutions (at 1/2) was carried out from a stock solution (10 mg/mL in methanol) of Acanthospermum hispidum extracts. The test sample (100 µL in methanol) was mixed with 200 µL of DPPH and then incubated at room temperature for 15 minutes. Absorbance was measured at 517 nm against a blank, using a spectrophotometer. The evaluation of the anti-radical properties of the sample made it possible to calculate the percentages of reduction of the radical DPPH• by decreasing the concentrations of the extracts. The experiment was carried out in triplicate (independent tests) and the concentrations, (µg/mL) trapping 50% of free radicals (IC50), were determined using the curves of the percentages of anti-radical activities as a function of the concentrations of the extracts.

1.6. Biomembrane protection

Desoxyribose degradation inhibitory assay

The hydroxyl radical is a far more reactive secondary free radical than its precursors (superoxide anion, hydrogen peroxide). The ability of the extracts to trap the hydroxyl radical was evaluated using the desoxyribose degradation scavenging assay, as

described by Perjési and Rozmer [13]. The hydroxyl radical is produced in situ by iron sulfate, which decomposes hydrogen peroxide into a hydroxyl radical. The hydroxyl radical that is formed attacks the desoxyribose (a component of the DNA) at the C2′ level and causes the opening of the ring with the formation of malonylaldehyde. This product, with thiobarbituric acid, forms a pink complex that is dosed at 532 nm. The reaction mixture consisted of 100 µL of the extract (1 mg/mL in a 50 mM phosphate buffer, pH 7.4), 100 µL of EDTA (1.04 mM aqueous), 100 µL of iron sulfate (100 µL aqueous mM), 100 µL of desoxyribose (60 mM aqueous), and 100 µL of hydrogen peroxide (10 mM). The volume was increased to 1 mL with a phosphate buffer, and then the mixture was incubated (37 °C for 1 hour). Trichloroacetic acid (1 mL, 15% aqueous) and thiobarbituric acid (1 mL, 0.675% in 25 mM aqueous NaOH) were added and the whole mixture was then incubated (100 °C for 15 minutes). After cooling in an ice bath (5 minutes), the tubes were centrifuged (3000× g for 10 minutes) and then 200 µL of the supernatant was transferred into 96-well microplates. The trapping of desoxyribose degradation was measured, using a spectrophotometer, at 532 nm against a blank. Gallic acid was used as a reference substance. The experiment was carried out in triplicate (independent tests) and the ability of the extract to trap desoxyribose degradation was expressed as a percentage of desoxyribose degradation trapping, which was compared to a control without the extract.

Lipid Peroxidation Inhibitory Assay

It is also important to evaluate the ability of a matrix to inhibit the initiation and the spreading of lipid peroxidation. The ability of *Acanthospermum hispidum* extracts to inhibit lipid peroxidation was evaluated using lecithin liposomes as a membrane model, according to the method described by Kumari et *al*. [14]. The reaction medium consisted of 100 µL of the extract (1 mg/mL in a 10 mM phosphate buffer, pH 7.4), 100 µL of iron sulfate (100 mM), 100 µL of EDTA (1.04 mM), 100 µL of hydrogen peroxide (10 mM), and 100 µL of an opalescent suspension of lecithin (10 mg/mL in a phosphate buffer). The reaction volume was increased to 1 mL with a phosphate buffer and then the mixture was incubated (37 °C for 1 hour). The reaction was halted by adding 1 mL of 0.25 N hydrochloric acid, containing 15% trichloroacetic acid and 0.675% thiobarbituric acid. The mixture was then heated to 100 °C for 15 minutes and cooled in an ice bath for 5 minutes. After centrifugation (3000× *g* for 10 minutes), the absorbance

of the supernatant was measured, using a spectrophotometer, at 532 nm against a blank. Gallic acid was used as a reference substance. The experiment was carried out in triplicate (independent tests) and the inhibition of lipid peroxidation by the extract was expressed as the concentrations (µg/mL) inhibiting the peroxidation of 50% of lipids (IC50). The IC50 values were determined by the percentage of inhibition curves as a function of the extract concentrations.

1.7. *In vivo* hepatoprotection

Animal conditioning

The experimental animals are male rats of Wistar variety weighing between 220-250 g. All animals were of FSPO sanitary status (free of specific pathogenic organisms).

Upon receipt, the rats are randomly placed in groups of 6 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 are kept in a constant temperature (22 ± 2) ° C pet shop subject to light/dark cycle of 12 /12 hours. All experimental animal protocols have been approved by the Institutional Animal Ethics Committee (SVCP / IAEC / I-020/2013-2014).

Animal treatment

The animals were distributed as follows:

Lot I (normal) receives water orally

Lot II (negative control) receives water orally

Lot III (positive control) receives silymarin, a reference antioxidant at the daily dose of 50 mg/kg of oral body weight.

The test lots (IV, V, VI, VII and VIII) receive a dose of the test fractions once a day (100 mg/kg body weight) respectively for the fractions ethyl acetate, dichloromethane, methanol aqueous and n-hexane. All treatments are administered orally. On day 7, with the exception of lot I, animals from the other lots received intraperitoneally diethylnitrosamine (200 mg/kg body weight). On the 8th day all rats were blood drawn by cardiac puncture and the liver removed.

Biochemical parameters

The biochemical parameters were determined from the serum obtained by centrifugation (3000 g, during 5 minutes) of the blood of the non-heparinized dry tubes. Alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), Bilirubin D, Bilirubin T, Protein T, Albumin, Alkaline phosphatase, Blood Glucose and Cholesterol were determined using a kit (LABKIT) according to the manufacturer's instructions. Creatinine was determined using the Spinreact kit following the manufacturer's instructions.

Assay of enzymatic antioxidants and non-enzymatic liver

The antioxidant activity of the extracts was therefore evaluated both by measuring the level of lipid oxidation, but also by measuring the activity of the enzymes involved in defending the body against oxidation phenomena. The level of MDA in the liver was measured according to Ohkawa [15]; SOD was tested using the standard method revealed by Misra and Fridovich [16]; Catalase was measured using a standard protocol given by Beers and Siezer [17].

1.8. Statistical evaluation

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 two values is significant when P=0.05.

2. RESULTS

2.1. phytochemistry

Determination of total phenolics

The determination of the total phenolics of the fractions showed varying content from 463.92 ± 10.51 to 2428.53 ± 5.77 mg EAG / g extract (Table 1). The ethyl acetate fraction contains most of total phenolic than the rest of the fractions.

Determination of total flavonoids

The total flavonoid assay confirms that the extracts contain a lot of flavonoids. The ethanolic extract has two fractions, namely the fraction of dichloromethane and that of

ethyl acetate with flavonoid contents relatively higher totals (47.76 ± 5.09 mg EQ/g extract and 41.21 ± 4.99 mg EQ / g). The amount of flavonoids varies from 11.71 ± 5.46 to 47.76 ± 5.09 mg EQ/g (Table 1).

Table 1: Results of total phenolics and total flavonoids

Extract	**Phytochemistry**	
	Total Phenolic (mg EAG/g extract)	Total flavonoids (mg EQ/g extract)
Hexane fraction	463.92±10.51^{d}	13.78±4.57^{e}
Dichloromethane fraction	731.69±1.52^{bc}	47.76±5.09^{a}
Ethyl acetate fraction	2428.53±5.77^{a}	41.21±4.99^{b}
Methanol fraction	856.78±5.36^{b}	21.03±2.33^{cd}
Aqueous fraction	1567.66±10.13^{ab}	11.71±5.46^{f}

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

3.2. *In Vitro* antioxidant activities

The antioxidant capacity of the extracts was evaluated according to three methods. Thus, it is first noted that with respect to the capacity of the trap extracts the DPPH is obtained and even lower inhibition concentrations with all fractions except the hexane fraction. The best 50 % inhibition concentrations were those of the ethyl acetate and dichloromethane fraction with 0.014 ± 0.002 and 0.02 ± 0.003 µg/mL, respectively (table 2).

Then the reduction power of the radical anion ABTS was evaluated. All fractions gave relatively lower activities than the ethyl acetate fraction with 256.54 ± 1.00 mM TEQ/g (table 2).

Finally, the iron ion reduction capacity with regard to the fractions, the ethyl acetate fraction reduces the iron ion better than the rest of the fractions (table 2).

Table 2: Results of Antioxidant Tests

Extract	Antioxydant Activities		
	DPPH Ic 50% (µg/mL)	ABTS (mmol TE/g)	FRAP (mg AAE/g)
Hexane fraction	0.08±0.001^{c}	123.18±1.52^{d}	49.62±7.49^{f}
Dichloromethane fraction	0.02±0.002^{b}	178.38±2.61^{b}	234.58±10.58^{d}
Ethyl acetate fraction	0.014±0.001^{a}	256.54±1.00^{a}	1220.14±2.34^{a}
Methanol fraction	0.022±0.002^{ab}	176.71±2.06^{c}	291.71±3.96^{cd}
Aqueous fraction	0.4±0.008^{d}	177.34±0.42^{bc}	190.51±3.90^{e}

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

2.2. Biomembrane protection

The protective power of the biomembranes of the extracts was evaluated with respect to their ability to inhibit the peroxidation of membrane lipids and the degradation of D-deoxyribose. On the other hand, among the fractions, those are the ethyl acetate and aqueous fractions which gave the best peroxidation inhibition concentrations of 50% of the lipids with respectively 20.45 µg/mL and 18.89 µg/mL (table 3). In a second step, it appears that concerning the inhibition of the degradation of D-deoxyribose that all the fractions showed a good activity for an initial concentration of 1 mg/mL. The best percentages of inhibition of the degradation are recorded at the level of the methanol fraction and that of ethyl acetate with respectively 86.46 ± 1.06 and 79.69% ± 1.015 (table 3).

Table 3: Results of inhibition of lipid peroxidation and inhibition of degradation of D-deoxyribose

Extract	Protection of the Biomembranes	
	Lipid peroxidation extract Ic 50% (µg/mL)	Degradation of desoxyribose inhibition (% Inhibition)
Hexane fraction	21.17±2.3^{b}	23.328±1.25^{e}

Dichloromethane fraction	24.67±1.7^{c}	64.97±0.02^{d}
Ethyl acetate fraction	20.45±3.1^{ab}	79.69±1.015^{b}
Methanol fraction	30±4.3^{d}	86.46±1.06^{a}
Aqueous fraction	18.89±1,2^{a}	Inactive
Gallic acid (reference)	42±1.8^{f}	84.68±3.31^{ab}

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

2.3. Hepatoprotective activity *in vivo*

Enzymatic and non-enzymatic antioxidants of the liver

Variation of catalase (CAT)

Results of the catalase assay for rat liver homogenates had shown that, compared to the negative control lot, there was a statistically significant increase in the amount of catalase for the lot that received the ethyl acetate fraction. The high dose of catalase was recorded in the liver homogenates of the rats treated with this fraction with a value of 0.235 ± 0.0038 U / mg protein (figure 1).

Variation of Superoxide Dismutase (SOD)

Treatment of rats with fractions of *Acanthospermum hispidum* at 100 mg / kg body weight showed an increase in SOD activity for liver homogenates. Thus, a significant difference was observed between the SOD activity of the negative control lot and that of the acetate, dichloromethane and methanol (P≥0.001) batches with respectively 0.338; 0.298 and 0.298 U/10mg of protein. In contrast, activity was impaired in negative control rats (DEN) with 0.016 ± 0.001 U/10 mg of protein (figure 1).

Variation of Malondialdehyde (MDA)

Compared with rats in the negative control lot, rats treated with the fractions of *Acanthospermum hispidum* showed a decrease in MDA levels. High peroxidation of fatty acids was observed in toxin-treated rats (DEN) with 0.0542 ± 0.001 × 100 µM/L (figure 1). On the other hand, there was no statistically significant difference between the MDA levels of the fractions even if we notice a considerable decrease in the level of MDA of the lot of the aqueous fraction (0.00702 × 100 µMol/L).

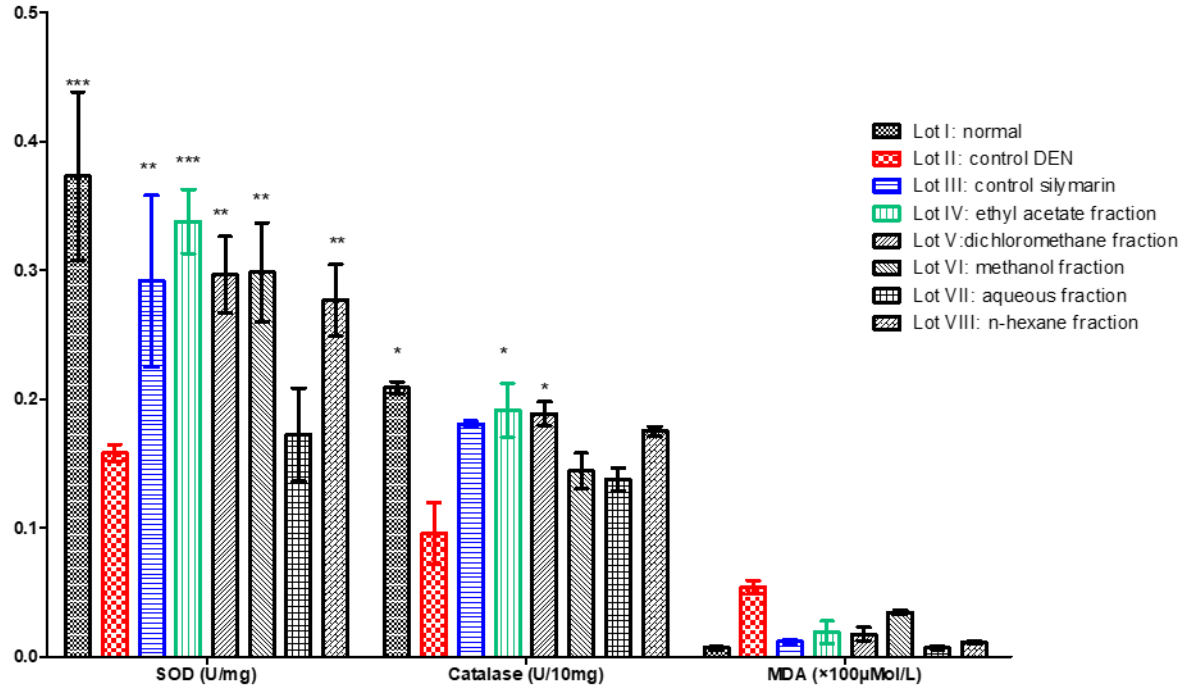

Figure 1: Variation of catalase, superoxide dismutase and malondialdehyde, $p > 0.05$: the difference is not significant; $0.05 > p > 0.01$: the difference is significant *; $0.05 > p > 0.001$: the difference is highly significant **;$p<0.001$: the difference is very highly significant***. Compared with the negative control (lot II).

Biochemical parameters

Assay for alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT)

The analysis of the parameters 24 hours after the intraperitoneal injection of a single dose of DEN showed a statistically significant increase in the serum ALAT levels of the animals treated with the fractions and those of the negative control lot. A high ALAT level of 253.6 ± 22.5 U/L was recorded for the control lot (lot II) and a relatively low level of 37.7 ± 11.2 U/L for the ethyl acetate fraction dose of 100 mg/kg body weight. Under the same conditions, the groups treated with the fractions had normal physiological values of serum ASAT. In contrast to ALAT levels, ASAT levels did not show a significant difference between those in the negative control lot and those in the lot that received the aqueous fraction (figure 2).

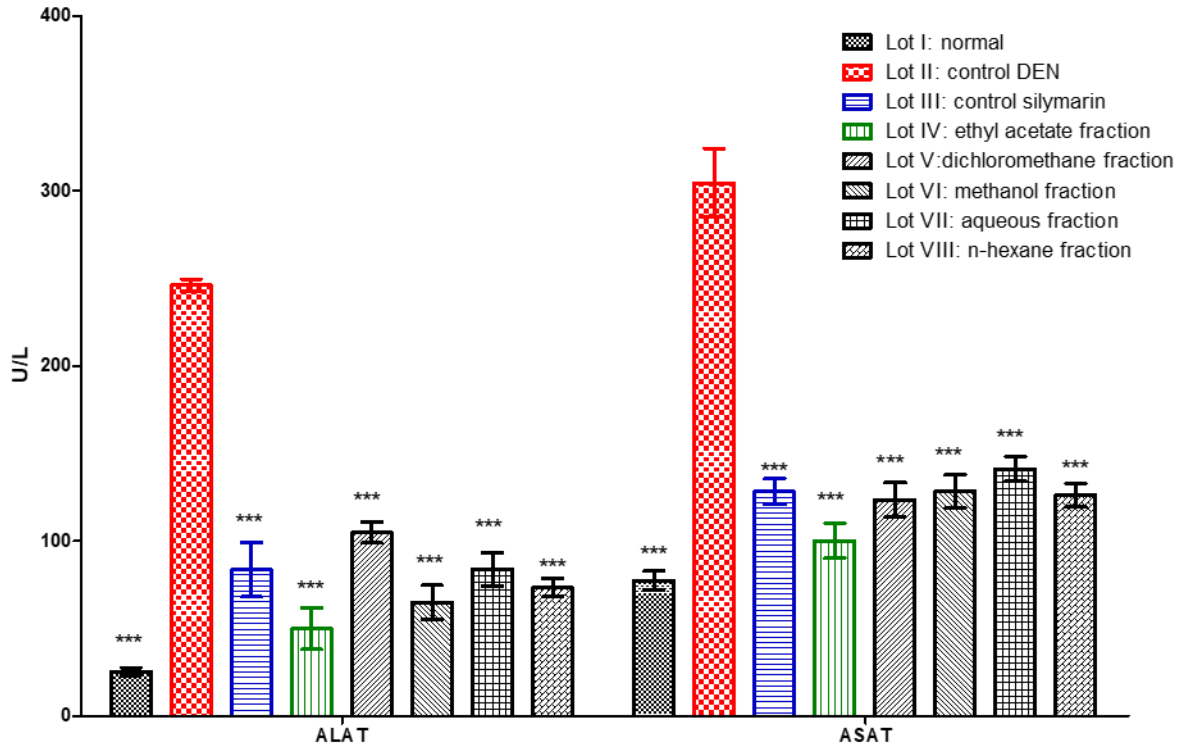

Figure 2: Assay Results of Alanine Aminotransferase (ALAT) and Aspartate Aminotransferase (ASAT), $p < 0.001$: the difference is very highly significant ***. Compared with the negative control (lot II).

Other biochemical parameters

The results of the other biochemical parameters show a significant difference between the animals of the negative control and those treated with *Acanthospermum hispidum* extracts at the following parameters: direct bilirubin, total bilirubin, total protein, alkaline phosphatase, blood sugar, creatinine and cholesterol.

A significant difference ($P<0.001$) was observed between the level of total bilirubin in animals treated with DEN alone (7.18 ± 0.93 U/L) and those treated with the ethyl acetate fraction (100 mg/kg body weight) of the ethanolic extract of *Acanthospermum hispidum*.

It was also found that the amount of cholesterol in the sera of the animals treated with hepatotoxin alone is higher (0.80 ± 0.07 g/L) compared to that of the animals treated with the fractions (figure 3 and 4).

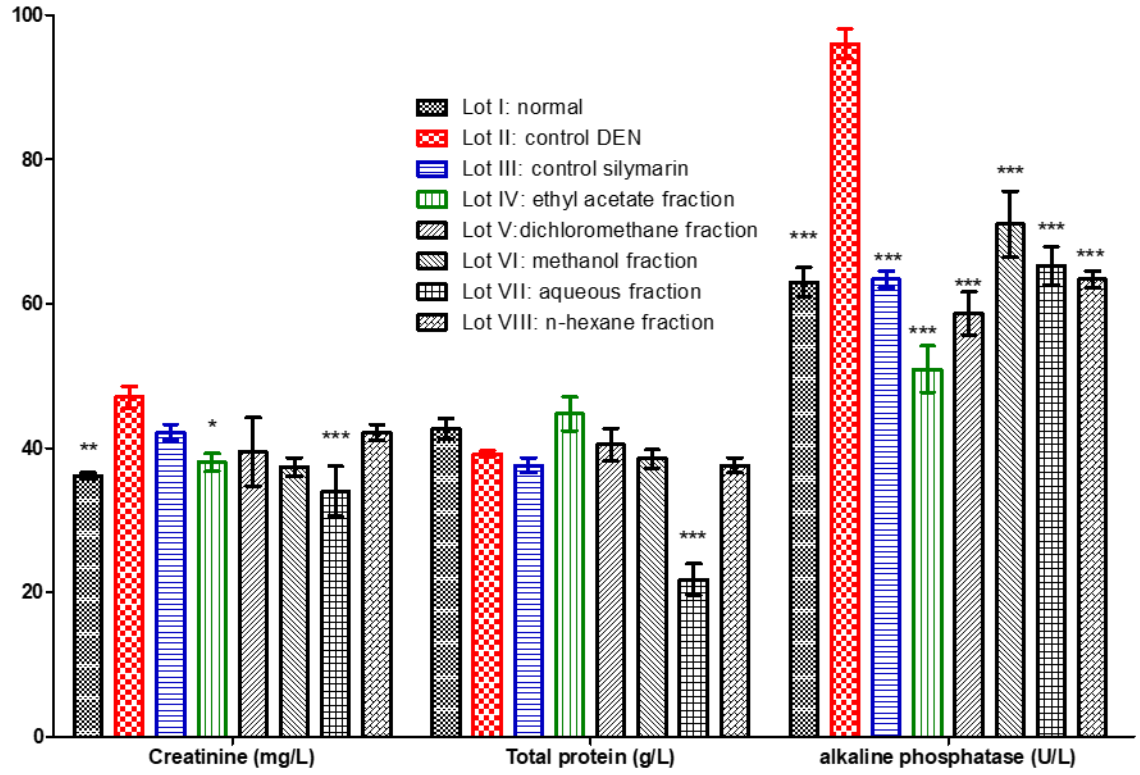

Figure 3: Results of certain biochemical parameters (creatinine, total protein, alkaline phosphatase)

The values are given on average ± Standard deviation. Student's test: p> 0.05: the difference is not significant ns; 0.05> p> 0.01 = the difference is significant *; p <0.001 = the difference is very highly significant ***. Compared to the negative control (lot II).

The level of blood glucose obtained between the test lots and that of the negative lot showed a significant difference (P<0.01). This difference would show that in animals pretreated with the fractions, the blood glucose level is regulated both in the animals that received the DEN alone, the value deviates much from the normal (0.55 ± 0.22 g/L) (figure 4).

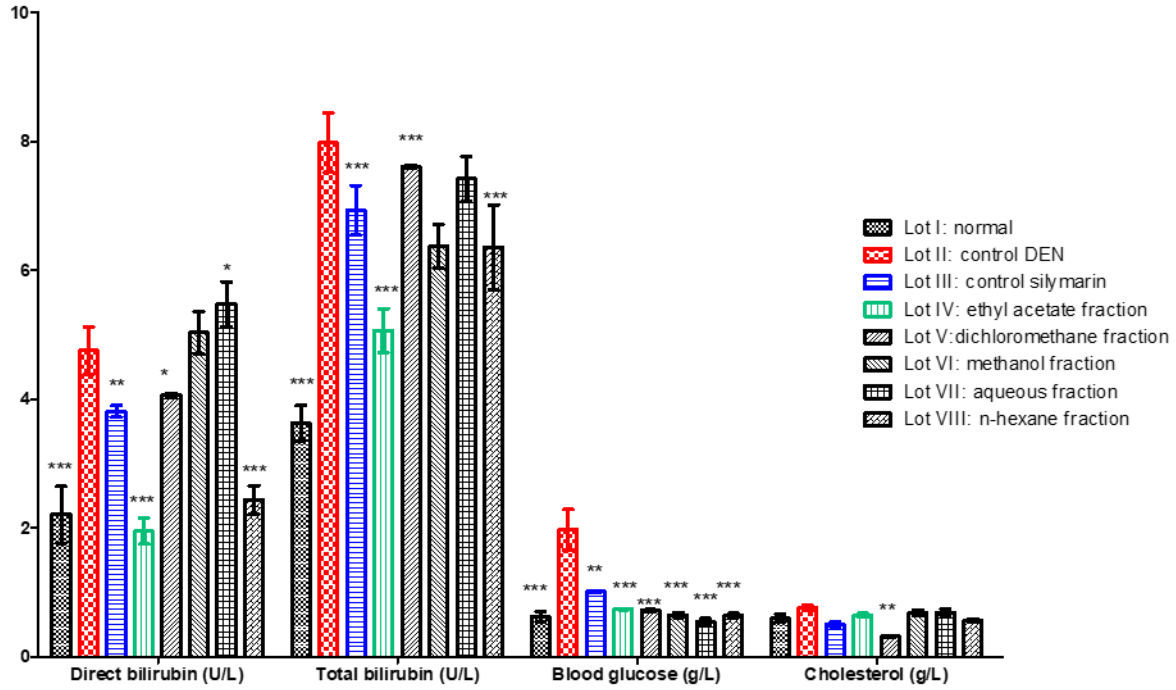

Figure 4: Results of certain biochemical parameters (Direct bilirubin, total bilirubin, blood glucose, cholesterol)

The values are given on average ± Standard deviation. Student's test: p> 0.05: the difference is not significant ns; 0.05> p> 0.01 = the difference is significant *; p <0.001 = the difference is very highly significant ***. Compared to the negative control (lot II).

3. DISCUSSION

From the analysis of the results on antioxidant tests (ABTS, DPPH and FRAP) had demonstrated that all the extracts had antioxidant activity. Thus it is found that the dichloromethane, ethyl acetate, methanol and aqueous fractions gave a relatively interesting DPPH radical inhibition capacity relative to quercetin with 0.044 µg/mL (reference compound). Similarly, the reducing power of the iron ion (FRAP) of the ethyl acetate fraction is more important. The best antioxidant properties of the ethanolic extract of the whole plant of *Acanthospermum hispidum* were also highlighted by Gomathi et *al*. [18]. The ethyl acetate fraction has a good antioxidant capacity compared to the other fractions; this would be related to the nature of the solvent [19]. The antioxidant capacity observed could be justified by the high levels of phenolic

compounds and total flavonoids highlighted by the results of the assay tests. These compounds are known for their high antioxidant capacity [20].

The radicals formed initiate the lipid peroxidation of the biomembrane by electrophilic attack that propagates through the generated radicals [21], the consequence being the degradation of the membrane integrity. For example, extracts that have been shown to inhibit lipid peroxidation and deoxyribose degradation could either directly remove hydrogen peroxide by transforming it into a water molecule or exert a positive action on the ionic transmembrane movement necessary to maintain the integrity of the hepatocyte membrane [22].

The administration of plant extracts in a preventive mode resulted in a significant inhibition of the increase in transaminase levels. Changes in parameters such as direct bilirubin, total bilirubin, total protein, alkaline phosphatase, blood glucose, and cholesterol in lot II animals (DEN alone) may indicate a high degree of hemolysis of red blood cells. DEN poisoning [23] whose effect has been attenuated by fractions. Among the fractions, the ethyl acetate fraction gave a good value of the amount of cholesterol (0.60 ± 0.74) at the dose of 100 mg/kg of body weight. Thus a very low level of cholesterol would express acute hepatitis and show a failure in hepatic activity. Similarly, it was found that DEN would disrupt an essential function of the liver that is to regulate blood sugar [24] by allowing the storage of surplus sugar in the form of glycogen or triglyceride [25]. In cases of viral hepatitis, hepatocellular injury, toxic or ischemic liver injury, higher serum bilirubin levels are observed [26]. Thus, in animals that received DEN alone, the mean value of total bilirubin is 7.18 ± 0.93 U/L. The administration of the ethyl acetate fraction in a preventive mode resulted in a significant regulation of the increase in the level of transaminases and other parameters such as direct bilirubin, total bilirubin, total proteins, alkaline phosphatase, blood glucose and cholesterol compared to the negative control. This could be a solid argument that would confirm the hepatoprotective potential of this fraction derived from *Acanthospermum hispidum*.

4. CONCLUSION

In this work, phytochemistry, by determining total phenolic compounds and total flavonoids, showed that *Acanthospermum hispidum* extracts have very high levels of

total phenolics and flavonoids. These phenolic compounds are found to possess significant antioxidant properties through anti-free radical tests (DPPH and ABTS) and the iron ion reduction test (FRAP). The results show a significant anti-hepatotoxic activity of the fractions of ethanolic extract in general and especially the ethyl acetate fraction specifically. This effect results in the ability of extracts to neutralize the impact of hepatotoxin by the preventive mode. Administration of the ethyl acetate fraction protected the liver better against the aggression of DEN compared to silymarin (lot III). This may justify the use of *Acanthospermum hispidum* in the treatment of hepatitis in traditional medicine in Burkina Faso.

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