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Anti-Fibrotic Effects of *Calotropis procera* (Ait.) R.Br Roots Barks against Diethylnitrosamine-Induced Hepatic Fibrosis in Rats

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Abstract

Background: Liver diseases including chronic hepatitis, steatosis, fibrosis, cirrhosis and liver cancer are now a public health problem. In 2002, cirrhosis accounted for 27.63% of hepatobiliary diseases in Burkina Faso. In Africa and more particularly in Burkina Faso, the majority of the population (about 80%) uses medicinal plants for their primary health care. *Calotropis procera* (Ait.) R.Br (Apocynaceae) is a medicinal plant used in Burkina Faso in the treatment of liver problems. This work aims to evaluate the anti-fibrotic properties of *Calotropis procera* roots barks. **Methods:** The anti-fibrotic activity of the ethanolic extract of *Calotropis procera* roots barks was evaluated using diethylnitrosamine (DEN) to induce liver fibrosis in male Wistar rats. Serum biomarkers, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), Total protein, Albumin, ϒ-Glutamyl transferase (GGT) were evaluated and the activities of antioxidant enzymes (Superoxide dismutase and catalase) as well as the level of malonedialdehyde (MDA) and that of nitric oxide (NO) were determined in the liver homogenate. **Results:** The treatment of rats suffering from hepatic fibrosis with the ethanolic extract leads to a significant restoration of the biomarkers of the hepatic function in particular, AST, ALP, GGT, Albumin. The extract also causes a reduction in oxidative stress in the liver through a significant increase in the activity rate of the antioxidant enzymes Superoxide dismutase (SOD) and catalase accompanied by a significant drop in the rate of MDA and NO suggesting the anti-oxidant effect of extract. **Conclusion:** The results of the study show that

the ethanolic extract of the roots barks of *Calotropis procera* has anti-fibrotic properties.

Keywords

Hepatic Fibrosis, *Calotropis procera*, Anti-Fibrotic Properties, Burkina Faso

1. Introduction

Liver diseases including chronic hepatitis, steatosis, fibrosis, cirrhosis and liver cancer are now a public health problem [1]. Cirrhosis is the 12th most common cause of death worldwide [2]. According to recent studies conducted in 2016, it is estimated that around 10 million people have liver fibrosis and around 1.5 million people have silent cirrhosis in Europe [3]. In 2002, cirrhosis accounted for 27.63% of hepatobiliary diseases in Burkina Faso [4]. Hepatic fibrosis can be defined as a pathological scarring process resulting from chronic liver damage. It is the major complication of many liver diseases including chronic hepatitis B and C virus, autoimmune hepatitis, chronic hepatitis due to drugs, natural toxins (Aflatoxins), chemicals (xenobiotics), alcohol, radiotherapy, etc. [5]. The spread of these chronic liver diseases at the origin of hepatic fibrosis is linked to several factors including lack of awareness, strong demography and weak health systems, especially in developing countries where there is a lack of access to testing and care. Studies have shown that hepatic fibrosis partly results from an imbalance between the synthesis and degradation of the various constituents of the extracellular matrix in favor of its synthesis. The formation of the extracellular matrix which leads to the establishment of collagen occurs following inflammatory and immune responses via the intervention of mediators such as Kupffer cells, platelets and lymphocytes infiltrating the liver, the production of reactive oxygen species or pro-inflammatory cytokines such as TGF-β against chronic liver injury [6]. These mediators stimulate the activation of hepatic stellate cells (HSCs) which secrete different cellular signals mainly made up of enzymes such as plasmin, cathepsins, elastase, and especially matrix-metalloproteases (MMPs) responsible for the formation and accumulation of components (type I collagens, III and IV, fibronectin, laminin and proteoglycans) of the extracellular matrix [7] [8]. Oxidative stress which action results in a drop in antioxidant activity in body plays an important role in the occurrence of hepatic fibrosis through different pathways. During chronic liver inflammation, there is production of reactive oxygen species (ROS), malonedialdehyde (MDA), reactive aldehyde products of lipid peroxidation which are factors promoting and aggravating hepatic fibrosis through their potential activators of HSCs. In Africa and more particularly in Burkina Faso, the majority of the populations (about 80%) use medicinal plants for their primary health care [9]. *Calotropis procera* (Ait.) R.Br (Apocynaceae) is a medicinal plant used in the treatment of many diseases. In Burkina Faso, the plant is used in the treatment of liver problems, infections, sickle cell

disease, asthma, tumors, ulcers [10] [11]. In our recent studies, *Calotropis procera* roots barks ethanolic extract showed good anti-hepatotoxic activity against the toxic effects induced by diethylnitrosamine in rats [12]. In addition, this extract showed low acute toxicity in male and female mice [12]. Based on the results of these studies, it appears more than necessary to evaluate the anti-fibrotic activity of *Calotropis procera* roots barks ethanolic extract on an experimental animal model.

2. Materials and Methods

2.1. Plant Material

Calotropis procera (Ait.) R.Br root bark was collected northeast of Ouagadougou in August 2015 according to GPS coordinates (12˚25'28.2"N; 1˚28'0.06"W). They were dried under laboratory conditions away from the sun and pulverized into a fine powder by a blade grinder (Gladiator Est. 1931 Type BN 1 Mach. 40461 1083). The species has been authenticated by the herbarium of the UFR/SVT of Joseph Ki-Zerbo University. A specimen of the species was deposited there under the identification code ID-17033.

2.2. Chemical Reagent

Monobasic sodium phosphate (NaH_2PO_4), dibasic sodium phosphate (Na_2HPO_4), sodium chloride (NaCl), iron dichloride ($FeCl_2$), sodium hydroxide (NaOH), trichloroacetic acid (TCA), thiobarbituric acid (TBA), sylimarin, sodium carbonate (Na_2CO_3), epinephrine, ethanol, hydrogen peroxide (H_2O_2), sulfanilamide, phosphoric acid, N-1-naphthylethylenediamine, diethylnitrosamine (DEN) were obtained from Sigma-Aldrich. They were all of analytical grade. Diagnostic kits for estimation of serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), Total protein, Albumin, ϒ-Glutamyl transferase (GGT), were obtained from SpinReact, Spain.

2.3. Preparation of Extract

The dry powder of *Calotropis procera* root barks was mixed with 96% ethanol at the proportion of 10%. The mixture was then subjected to mechanical stirring for 24 h at ambient laboratory temperature. After 24 hours, the mixture was filtered and the extract obtained was concentrated using a rotary evaporator fitted with a vacuum pump and then ventilated to dryness in an oven at 40˚C for 48 hours before be stored in the refrigerator at 4˚C.

2.4. Animals

Male rats of Wistar strains with an average weight of 240 - 260 g were acquired from the MEPHATRA/PH animal facility and the UFR/SVT animal facility of the Joseph Ki-Zerbo University for this study. Male rats of Wistar were placed in groups in cages and then acclimatized two weeks before the start of the experiment. During the acclimatization period the animals were fed pellets and water

then kept under the conditions of temperature at 23˚C ± 2˚C, humidity at 60% ± 10% and light/dark cycle 12 h/12h.

2.5. Experimental Study

The rats were randomized into seven groups of ten.

Group I: normal control received 9‰ NaCl (vehicle used) intraperitoneally once a week for the first four consecutive weeks.

Group II (DEN control): received DEN intraperitoneally at a dose of 70 mg/kg of body weight (bw) once a week for the first four consecutive weeks to allow the onset of hepatic fibrosis.

Group III (sylimarin control): received DEN intraperitoneally at a dose of 70 mg/kg bw once a week for the first four consecutive weeks. They then received the sylimarin at dose of 50 mg/kg bw orally five (5) times a week for another four consecutive weeks.

Group IV, V and VI (tests groups): received DEN intraperitoneally at a dose of 70 mg/kg bw once a week for the first four consecutive weeks. They then received the extract respectively at doses of 50 mg/kg, 100 mg/kg and 200 mg/kg bw orally five (5) times a week for another four consecutive weeks.

Group VII: received nothing during the first four consecutive weeks but received the extract at a dose of 200 mg/kg bw orally under the same conditions as the test groups during the last four weeks to assess its effect on the various parameters to be determined.

After treatment with the extract, all rats were sacrificed. The rats were previously fasted the day before the sacrifice. After the sacrifice, the blood and the liver were taken for the various analyses.

2.5.1. Analysis of Physical Parameters

During the test, the weights of the rats were taken every two days to assess the impact of the toxin and the extract on the animals. Food and water consumption was also determined.

2.5.2. Analysis of Biochemical Parameters

Hepatic fibrosis is a scarring process causing the parenchymal tissue of the liver to be replaced by the extracellular matrix formed by collagen fibers. Hepatic fibrosis is characterized by collagen formation, an increase in serum of certain biochemical parameters (AST, ALT, ALP, Albumin, Prothrombin, Platelets) and a drop in GGT activity. For this purpose, the blood collected was centrifuged at 3000 g for 15 min then the serum was collected for the analysis of serum markers of fibrosis (AST, ALT, ALP, GGT, Total proteins, Albumin) through kits.

2.5.3. Antioxidant Enzyme Activities *in Vivo*

Part of the sampled liver is ground to a proportion of 10% in PBS buffer. The homogenate obtained is centrifuged at 12,000 g for 15 min then the supernatant is sampled to evaluate the enzymatic and non-enzymatic antioxidant activities.

1) Superoxide dismutase (SOD) activity

SOD activity was determined by the standard method disclosed by Misra *et al.*, [13]. It is based on the inhibition of epinephrine-adrenochrone transition by the enzyme. The reaction medium comprises 0.5 mL of liver homogenate supernatant, 0.5 mL of distilled water to dilute the sample, 0.25 mL of ice-cold ethanol and 0.15 of chloroform was added to precipitate the reaction mixture. The reaction mixture is stirred well for about 5 minutes at 4˚C then centrifuged. The adrenochrone produced in the reaction mixture contains 0.2 mL of EDTA (0.6 mM), 0.4 mL of Na_2CO_3 (0.25 M) and 0.2 mL of epinephrine (3 mM), the volume final was adjusted to 2 mL with distilled water (0.3 mL) then absorbance readings were measured at 420 nm in a BioteckEpoch spectrophotometer. The transition from epinephrine to adrenochrome was determined by adding the required amount of enzyme to assess enzyme activity expressed in terms of unit/min/mg protein.

2) Catalase activity

Catalase activity was determined using the standard method given by Beers and Siezer [14]. The degradation of H_2O_2 by addition of the enzyme is followed by absorption of light. The absorption of the peroxide solution in the UV region is determined. The reaction medium comprises 1.9 ml of phosphate buffer (0.05 M, PH 7), 1.0 ml of H_2O_2 substrate (30 mM) and 0.1 ml of liver homogenate supernatant. Activity was measured as a change in optical activity over a density of 240 nm at one minute intervals for approximately 3 minutes. Catalase activity was expressed in terms of µmol H_2O_2 consumed/min/mg protein.

3) Thiobarbituric acid-reactive substance (TBARS) assay

The TBARS assay in extracts or fractions of extracts was determined by the 2-thiobarbituric acid method [15]. This method measures colorimetrically the reaction product of thiobarbituric acid with malondialdehyde (MDA), a by-product of oxidized lipids (red complex). The reaction medium consisted of 1.0 mL of liver homogenate (10%), 200 µL of PBS buffer (0.1 M; pH 7.4), 50 µL of $FeCl_2$ (0.5 mM) and 50 µL of H_2O_2 (0.5 mM). The mixture was then incubated at 37˚C for 60 minutes, then 1 mL of trichloroacetic acid (TCA) (15%) and 1 mL of 2-thiobarbituric acid (TBA) (0.67%) were added and the mixture is heated in a water bath at 100˚C. for 15 minutes. Absorbances were read at 532 nm using the BioteckEpoch spectrophotometer. The inhibition of lipid peroxidation is measured through the quantification of the malonedialdehyde formed which is expressed in µmol MDA/mg of protein.

4) Determination of NO

Nitric oxide was determined in rat liver homogenate using the quantitative colorimetric method based on Griess's reagent [16]. Briefly, 150 µL of liver homogenate supernatant was mixed with 150 µL of Griess' reagent (1 g/L sulfanilamide, 25 g/L phosphoric acid and 0.1 g/L N-1-naphthylethylenediamine). The mixture was then incubated at room temperature for 10 minutes. Absorbance was read at 540 nm into BioteckEpoch spectrophotometer against a standard curve that was made with sodium nitrite dissolved in distilled water (20 - 100 µg/mL). The results were expressed in µg NO/g of liver.

2.6. Statistical Analysis

Statistical analysis was carried out with GraphPad Prism software (version 5.0) the means and the deviations standards. ANOVA one way or two way followed by the Bonferroni test was used to measure the degree of statistical significance of the results. A significant difference is considered for $P < 0.05$.

3. Results

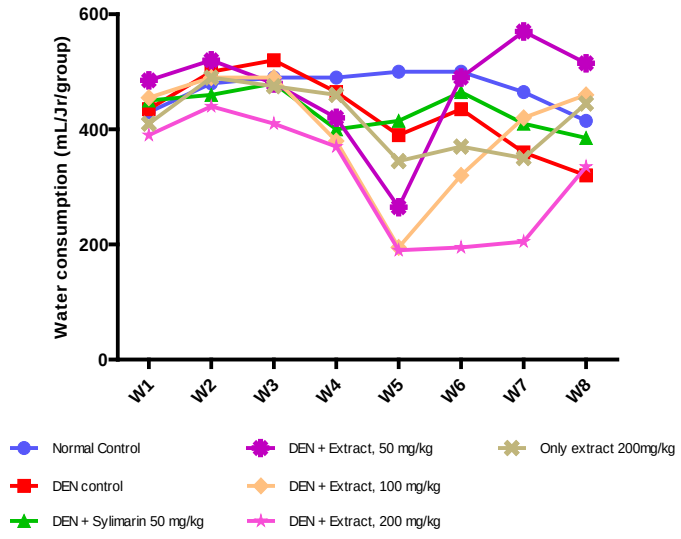
3.1. Water and Food Consumption

Figure 1(a) and Figure 1(b) respectively show the effects of extracts and/or DEN on water consumption and food consumption in rats of the different groups during the anti-fibrotic test. From the 1st week to the end of the 4th week, it appears that the consumption of water and food remained constant for the rats of normal control and those in rats of the group having received only the extract. Concerning the other groups which received the DEN, the consumption of water and food remained constant until the end of the 3rd week but they drop slightly from the beginning of the 4th week until the end of the 4th week; this suggests the pathological state of animal organisms, *i.e.* the onset of hepatic fibrosis in rats. From the beginning of the 5th week until the end of the 8th week, food and water consumption remained constant for normal control and sylimarin control. Sylimarin therefore has no major impact on food and water consumption in rats. However, there is a continuous slight decrease in food and water consumption in DEN control rats suggesting the progressive evolution of hepatic fibrosis. The results also show a significant drop in food and water consumption in the rats of the groups which received the extract from the beginning of the 5th week, *i.e.* the 1st week of administration of the extract. This could be explained by the pathological state of these animals and/or by the fact that extract contains anorectic compounds, as evidenced by this drop in food and water consumption in rats of the group having received only the extract. From the 6th week, there was a gradual resumption of food and water consumption in rats of the groups which received the extract; this would be explained on the one hand by the fact that the effect of extract would lead to a regression of hepatic fibrosis and on the other hand by the fact that the organisms of animals such as those of group having received only the extract would have developed physiological mechanisms at the over time to inhibit the anorectic effect of the extract.

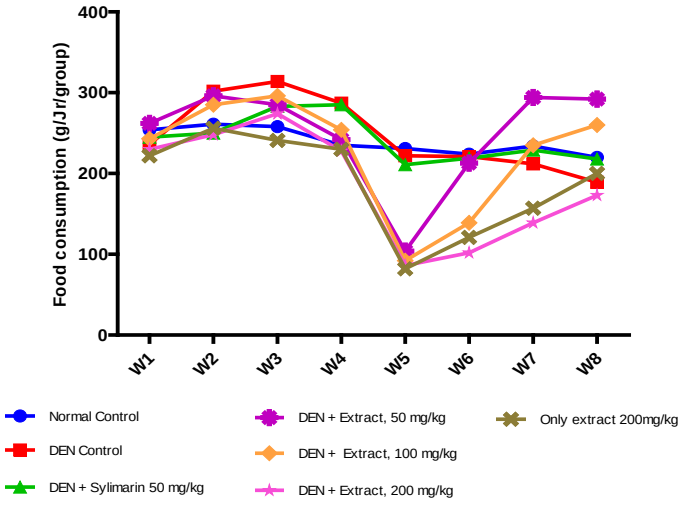
3.2. Evaluation of Weight in the Rats: Weight Gain

Figure 2 shows the weight gain in the rats of the different groups during the anti-fibrotic test. During the first four (4) weeks corresponding to the period of administration of the DEN, there was a weight gain in the rats of all the groups. These results could be explained by the fact that water and food consumption during this period remained constant. However, by comparing the weight gain in the normal control group with those who received DEN during this same period, there is a weight gain of about 25 g more in the normal control group

compared to the DEN control group, suggesting the pathological state of animal organisms, that is to say the non-negligible toxic effect of DEN on rats. During the last four weeks corresponding to the period of administration of the extracts in the rats of test groups and group having received only the extract, there is a significant drop in weight in the rats of these groups. This could be explained by the drop in water and food consumption (**Figure 1(a)** and **Figure 1(b)**) which could be due to the presence of anorectic compounds in the extract, as evidenced by the drop in weight in rats of group having received only the extract. There is, however, a weight regain from the 6th week in the rats of groups IV and V which respectively received the doses of 50 and 100 mg/kg bw of extracts. However, a progressive weight gain in the rats of the normal control, DEN control and sylimarin control groups is recorded.



(a)



(b)

Figure 1. Water (a) and Food (b) consumption of rats per group during the anti-fibrotic test.

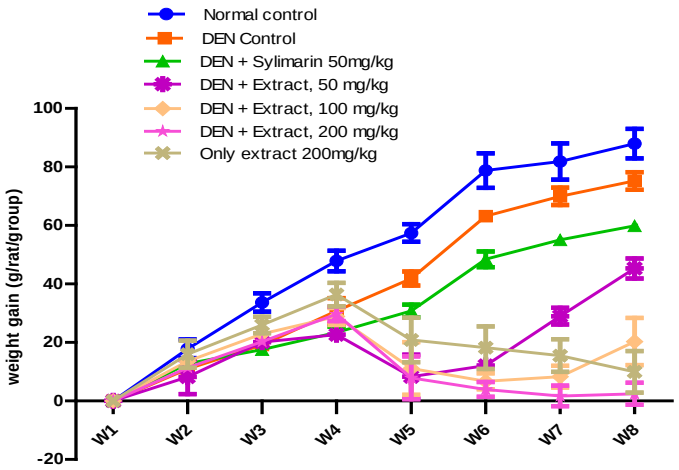


Figure 2. Weight change during the anti-fibrotic test. Values are mean ± standard deviation (n = 10).

3.3. Relative Liver Weight

The relative weight of the liver which is the percentage obtained from the ratio between the weight of the liver and the weight of the rat, makes it possible to assess the anti-fibrotic effect of the extract. The statistical analysis of the relative weights of the livers of each group treated with the ethanolic extract or the sylimarin shows a statistically significant difference compared to the relative weight of the livers of the DEN control group ($P < 0.05$). There is an increase in the relative weight of the livers in the treated groups compared to the DEN control group (**Figure 3**), suggesting that the extract would lead to a regression of hepatic fibrosis through its stimulatory effect in the regeneration of hepatic tissue.

3.4. Effects of the Extract on Biochemical Parameters

3.4.1. Evaluation of ALT, AST and PAL Levels in Serum

Figure 4 shows the results of the biochemical parameters ALT, AST and ALP. These results show that there is no statistically significant difference in the level of alanine aminotransferase, a liver-specific parameter, in the groups treated with the extract and sylimarin compared to the DEN control group. However, there is a significant decrease in AST and ALP in the groups treated with the extract at different doses compared to the DEN control group ($P < 0.001$). Groups IV and VI, which received doses of 50 and 200 mg/kg bw respectively, presented the best ALP levels with 165 ± 16.01 U/L and 167 ± 7 U/L respectively. The results also show that the extract has no negative effects on these parameters as evidenced by the group which received only the extract alone.

3.4.2. Evaluation of Total Protein and Albumin Levels in Serum

Figure 5 represents the evaluation of total proteins and albumin in rats treated with the ethanolic extract. At the level of total proteins, there is no significant difference between the rats of groups treated and those which received only DEN except for group III, a sylimarin control which showed a significant increase in the level of total proteins. With regard to albumin, which is an important fibrot-

ic marker, there is a significant increase in this parameter in the treated groups compared to the DEN control group ($P < 0.05$). The extract has no negative effects on these two parameters, as evidenced by the group having received the extract alone.

3.4.3. Evaluation of Ɣ-Glutamyl Transferase (GGT) Level in Serum

The effect of the ethanolic extract on the variation in the rate of GGT is presented in Figure 6. The evaluation of GGT shows a significant drop in this parameter in the rats of the groups pretreated with the sylimarin and the ethanolic extract at different doses compared to the rats of the DEN control group ($P < 0.01$). The best GGT activity was obtained with the dose of 200 mg/kg bw (0.4 ± 0.54 U/L) which is significantly lower compared to that of the DEN control group (3.50 ± 0.55 U/L) ($P < 0.001$). The group having received the extract alone presented a significantly low GGT activity compared to the DEN control group, suggesting that the extract has no negative effects on this parameter ($P < 0.001$).

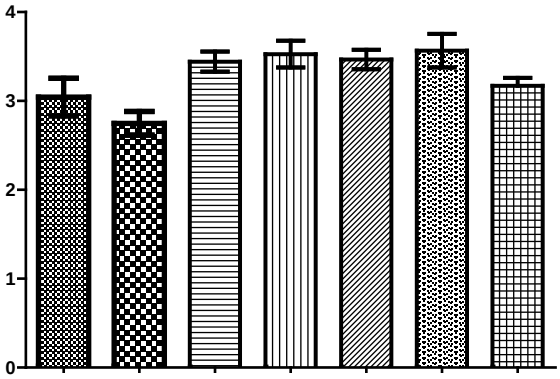


Figure 3. Relative weight of livers (%) of rats (n = 10). * $P < 0.05$, significant difference compared with DEN control.

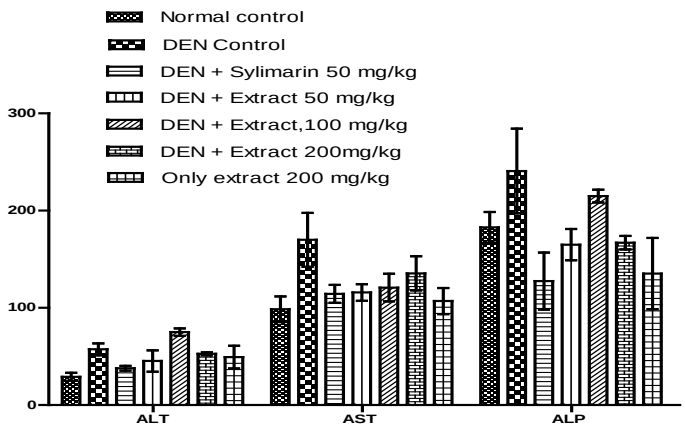


Figure 4. Effects of ethanolic extract on ALT, AST, ALP parameters. Values are mean ± standard deviation (n = 10). Anova two way, Bonferroni test: *** $P < 0.001$, very highly significant difference compared with the DEN control; ** $P < 0.01$, highly significant difference compared with DEN control; * $P < 0.05$, significant difference compared with DEN control.

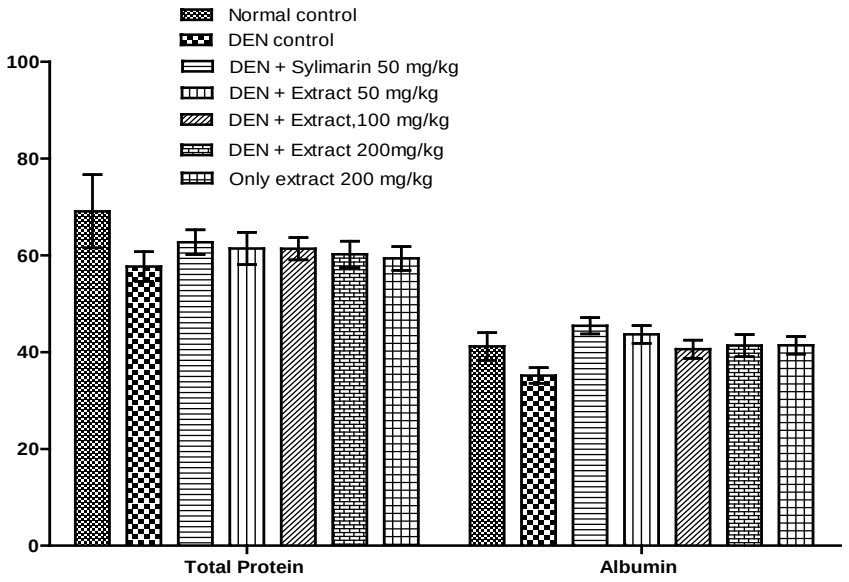


Figure 5. Effects of ethanol extract on total protein, albumin. Values are mean ± standard deviation (n = 10). Anova two way, Bonferroni test: *** P < 0.001, very highly significant difference compared with the DEN control; ** P < 0.01, highly significant difference compared with DEN control; * P < 0.05, significant difference compared with DEN control.

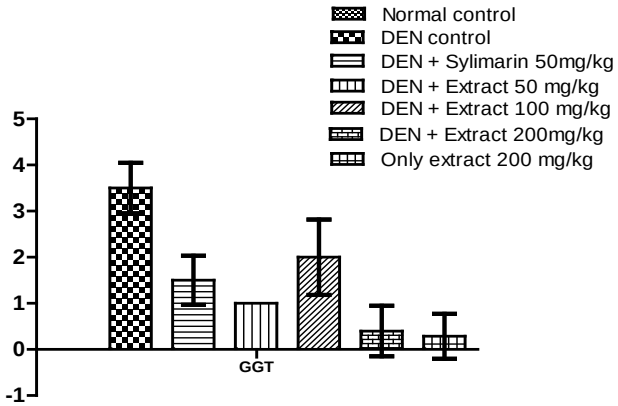
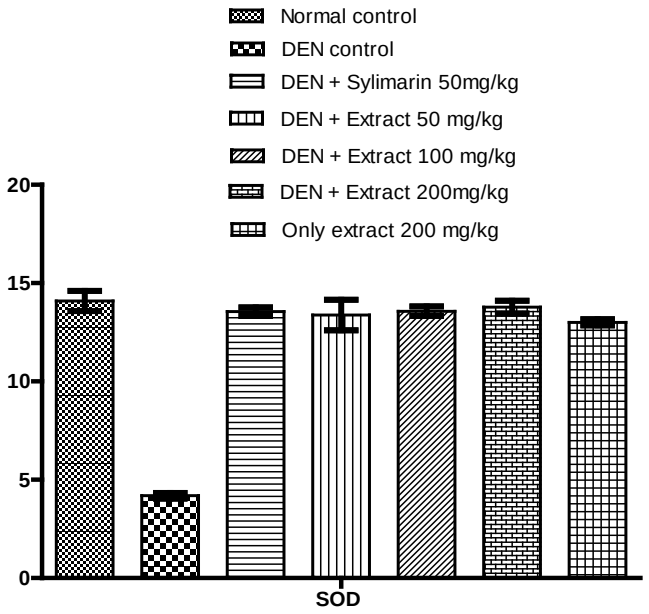


Figure 6. Effects of ethanol extract on ϒ-Glutamyl Transferase (GGT). Values are mean ± standard deviation (n = 10). Anova two way, Bonferroni test: *** P < 0.001, very highly significant difference compared with the DEN control; ** P < 0.01, highly significant difference compared with DEN control; * P < 0.05, significant difference compared with DEN control.

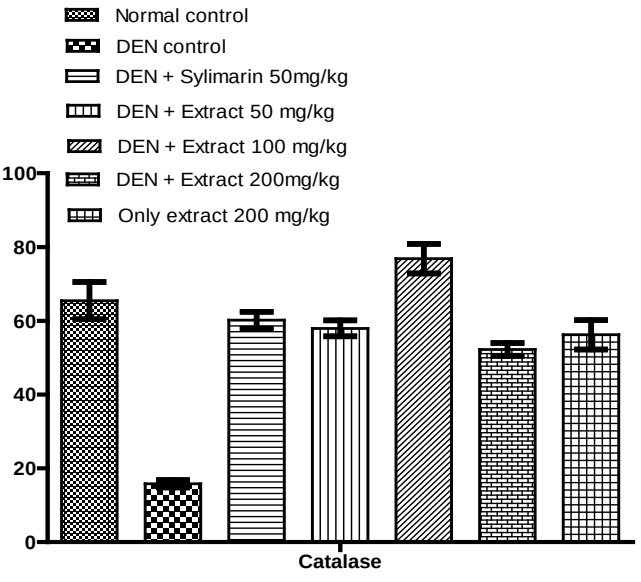
3.5. Evaluation of Antioxidant Activities *in Vivo*

Figure 7(a) and Figure 7(b) show the evaluation of the activities of the antioxidant enzymes SOD and Catalase in rats. This evaluation reveals a significant increase in the activities of the antioxidant enzymes SOD and catalase in the treated groups compared to the DEN control group (P < 0.01). The best catalase activity (76.86 ± 13.84 nmol SOD/mg protein) was recorded with the dose of 100 mg/kg bw. These results show that the extract stimulates the production of anti-

oxidant enzymes which are powerful regulators of oxidative stress. The evaluation of the MDA (Figure 7(c)) and NO (Figure 7(d)) levels showed a significant reduction in each of these parameters in the treated groups compared to the group that received only DEN ($P < 0.001$). This shows that the ethanolic extract protects on the one hand the cell membranes of the hepatocytes against the attacks linked to the hepato-toxicity of DEN during chronic inflammation of the liver or liver fibrosis and on the other hand that this extract also leads to a decrease in NO production in the liver. Overall, it appears that the dose of 200 mg/kg bw has the best antioxidant activity in vivo. The results also show that the extract alone has no negative effects on these four parameters.



(a)



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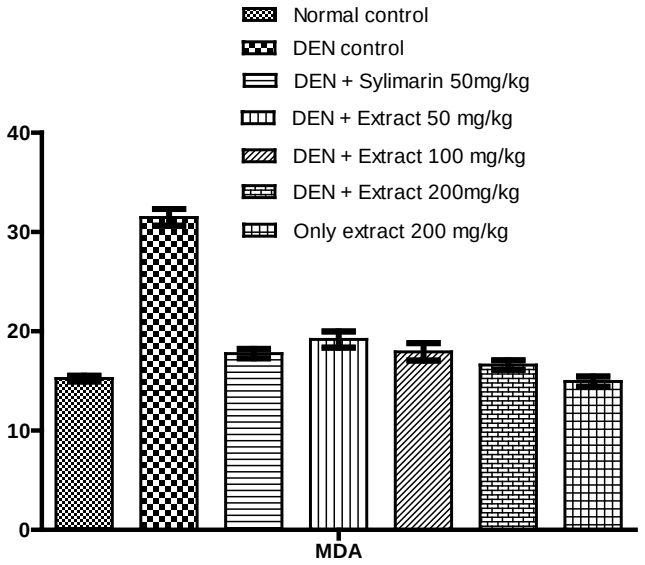

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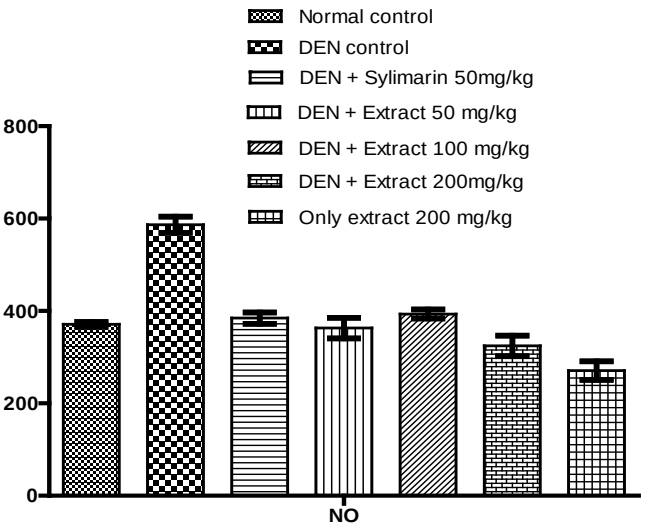

(d)

Figure 7. Effects of extract of *Calotropis procera* on SOD (a), Catalase (b), MDA (c), NO (d). Values are mean ± standard deviation (n = 10). Anova two way, Bonferroni test: *** P < 0.001, very highly significant difference compared with the DEN control; ** P < 0.01, highly significant difference compared with DEN control; * P < 0.05, significant difference compared with DEN control.

4. Discussion

Chronic liver inflammation is the main risk factor for liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Several studies have shown the major role of stellate cells in the development of hepatic fibrosis [17]. The activation of these cells by mediators such as kupffer cells, initiates the formation of the extracellular matrix which is transformed into collagen causing hepatic fibrosis, the effect of which is reversible [18]. Serum markers, ALT, AST, ALP, GGT, total protein,

albumin are important physiological parameters in the diagnosis of liver dysfunction. To some extent, the values of total proteins and albumins reflect the ability of the liver to synthesize proteins suggesting their fundamental role in the regenerative process of hepatocytes while the values of ALT, AST, ALP, and GGT reflect liver damage [19]. Hepatic fibrosis and/or hepatic cirrhosis induced by DEN in rats leads to an increase in the level of serum biomarkers, ALT, AST, ALP and a decrease in the level of total proteins and albumin [20] [21]. The treatment of rats with the ethanolic extract did not show a statistically significant difference for the specific liver parameter ALT in the treated groups compared to the control group DEN. This could be explained by the fact that the ALT level decreases with the development of fibrosis [22]. Gamma glutamyl transferase is a serum biomarker present in the cell membranes of many tissues, but it is much more present in the hepatobiliary system [20]. It is an important biomarker in the diagnosis of early liver fibrosis [23]. Its level increases during hepatic fibrosis induced in rats by diethylnitrosamine [20]. However, the treatment of rats with the ethanolic extract leads to a significant drop in this parameter. This shows that the extract has an anti-fibrotic effect. Albumin is an essential serum marker of hepatic fibrosis and/or hepatic cirrhosis [24], which is mainly produced in the liver. Its rate drops considerably during hepatic fibrosis or liver cirrhosis. The treatment of rats with the ethanolic extract at different doses leads to a significant increase in albumin levels compared to rats in the DEN control group. This suggests that the ethanolic extract contains compounds that stimulate the production of albumin which activity could promote the degradation of collagen and the regeneration of hepatocytes. The ethanolic extract could therefore be a therapeutic means of hepatic fibrosis. Studies have reported that long-term administration of human albumin-rich serum to a patient with hepatic cirrhosis clears the patient's ascites and results in regression of cirrhosis [25]. In addition, albumin acts as an anti-inflammatory substance by directly preventing the expression of tumor necrosis factor α (TNFα) transcription and indirectly by preserving cellular glutathione which would thus protect cells against oxidative damage [24]. It also blocks the activation pathway of the pro-inflammatory transcription factor NF-kB by preventing the expression of TNFα [24].

The increased production of reactive oxygen species is a common factor in all types of hepatitis and it constitutes an important indicator of the pathogenesis of chronic hepatitis and hepatic fibrosis. Liver inflammation accelerates oxidant production and decreases antioxidant defense capacity, promoting the onset of oxidative stress and associated tissue damage that progresses to fibrosis if oxidative stress is not controlled [26]. Oxidative stress could be used to provide a therapeutic pharmacological response to chronic hepatitis and liver fibrosis. Antioxidants are natural or synthetic compounds whose main function is to fight against oxidative stress [27], by delaying or preventing the oxidation of substrates, such as lipids, proteins, deoxyribonucleic acid (DNA), by lowering oxidative stress, DNA mutations, malignant transformations, as well as other cellular damage parameters [28]. Due to the role of hepatocytes in the metabolism of

drugs, xenobiotics, these cells become the target of reactive oxygen and nitrogen species, yielding reactive oxygenated metabolites, thus requiring a defense system against significant oxidant [29]. The endogenous antioxidant system, made up of enzymes such as superoxide dismutase, catalase and glutathione peroxidase, has the main role of neutralizing ROS by transforming them into stable and non-reactive molecules [26]. SOD and catalase activities show that the ethanol extract contains molecules that can modulate the production of antioxidants in hepatocytes. Thus, these molecules would stimulate the production of antioxidant enzymes (SOD and catalase) in order to neutralize the oxidants which constitute one of the mechanisms of activation and proliferation of stellate cells at the origin of the synthesis of the extracellular matrix (ECM). This suggests that the extract would prevent or attenuate the development or progression of hepatic fibrosis and cirrhosis by deactivating liver stellate cells [30]. Nitric oxide is a biologically active radical synthesized by the enzyme nitric oxide synthase (iNOS) in the liver. The increased activity of iNOS in hepatic cells leads to a high level of NO production and increased oxidative stress due to the formation of peroxynitrite (ONOO) which is a very reactive, toxic and oxidizing compound, inducer of peroxidation lipid and DNA damage [20]. Peroxynitrite causes the production of fibrogenic cytokines which stimulate the activity of stellate cells causing the synthesis of collagen [31]. Administration of the ethanolic extract of *Calotropis procera* roots barks to rats with hepatic fibrosis leads to a reduction in the amount of NO produced, reflecting that the extract would have an inhibitory activity of iNOS. This suggests that extract has anti-fibrotic properties that protect the liver against the toxic effect from NO.

Mediators such as MDA/4-HNE, cytokines, ROS/RNS and hepatotoxins that are released after hepatic damage are potential activators of liver star cells, causing the genes expression of procollagen [32]. MDA, which contributes to liver inflammation by activating the NF-kB factor, is also a pro-fibrotic stimulus that leads to an increase in the expression of tissue inhibitor genes of TIMP1 and procollagen regulators [33]. In addition, a strong correlation was found between the MDA index produced during lipid peroxidation of the liver and the hepatic fibrosis score in patients with chronic hepatitis C, demonstrating that MDA adducts play a major role in the pathogenesis of liver fibrosis [20]. Treatment with ethanolic extract of DEN-induced hepatic fibrosis in rats leads to a significant decrease in MDA levels, suggesting its anti-fibrotic potential. Phytochemical screening of the ethanolic extract of *Calotropis procera* root bark showed that it contains steroids, terpenoids, cardenolides, saponosides but also phenolic compounds in the form of traces [12]. The evaluation of the compound contents of the extract showed that it is mainly made up of triterpene phytoconstituents [34]. Triterpenes, particularly pomolic acid, significantly inhibit the viability of stellate cells (HSC) in the liver, the activation of which leads to the production of collagen [35]. The extract is said to contain anti-fibrotic compounds that may cure or alleviate liver fibrosis.

5. Conclusion

This study showed that the ethanol extract of *Calotropis procera* roots barks has anti-fibrotic properties against hepatic fibrosis induced by diethylnitrosamine in rats with the best dose being that of 200 mg/kg bw. The results of the study thus show that the extract contains compounds capable of reversing liver fibrosis thanks to its antioxidant and regenerative effects on liver tissue. The extract could be used as a supplement in the treatment of hepatic fibrosis for its stimulatory on albumin production and regulator effects of oxidative stress.

Ethical Approval

The experiment was carried out in strict compliance with the instructions of the Institutional Animal Ethics Committee on the protection of animals used for scientific purposes according to the international standards set by the European Union (2010/63/EU). Code of ethical approval: 2010/63/UE, date of approval: October 20, 2010.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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