

(RESEARCH ARTICLE)



Preliminary phytochemical and hepatoprotective studies of ethanol extract of *Desmodium repandum* (Vahl) D.C. in paracetamol induced liver injury in guinea pigs

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Abstract

Medicinal plants are used worldwide in folk medicine to treat many diseases, including those affecting the liver, but most of those plants are not yet studied. The present study aimed at the in vivo evaluation of the hepatoprotective potential and doing a preliminary phytochemical screening of *Desmodium repandum* (Vahl) D.C. leaves ethanolic extract, a plant used in many regions of Africa to treat liver diseases. The presence of main phytochemical groups was assessed by using specific reactions of identification. Hepatoprotective activity was evaluated in male guinea pigs; the hepatotoxicity was induced by oral administration of paracetamol (acetaminophen) at the dose of 500 mg/kg. Silymarin was used as the reference drug at the dose of 100 mg/kg, the control group received a solution of carboxymethylcellulose (CMC) 1%, and the experimental groups 100 and 200 mg of the extract per kg of body weight. The results of phytochemical screening revealed the presence of anthocyanin, phenolic compounds, coumarins, flavonoids, quinones, tannins, sterols, and triterpenes. At the dose of 200 mg/kg, the extract reduced significantly ($p < 0.05$) the blood concentration of aspartate transaminase (AST), alanine transaminase (ALT), and total bilirubin after intoxication by paracetamol comparatively to the control group. This work demonstrated for the first time the hepatoprotective potential of the ethanolic extract of *Desmodium repandum* (Vahl) D.C. leaves.

Keywords: *Desmodium repandum* (Vahl) DC; Phytochemical screening; Hepatoprotective; Paracetamol; Liver

1. Introduction

The liver is the second-largest organ of the human body after the skin and one of the essential organs. It serves various crucial functions, including metabolic, vascular, immunological, secretory, and excretory processes. It is also a real organ in the detoxification of the human body [1–3]. But the ability of the liver to perform these functions is often compromised by numerous exogenic substances the human body is exposed to. Hepatic diseases are among the most critical world public health problems [2]. The term hepatic disease refers to any harm to the liver structure (tissues and cells) and liver function that can be induced by biological factors (bacteria, virus, and parasites) and autoimmune diseases (immune hepatitis, primary biliary cirrhosis), as well as by the action of different xenobiotics, such as toxic compounds (carbon tetrachloride (CCl₄), thioacetamide, dimethylnitrosamine (DMN), etc.). Excessive alcohol consumption and some drugs such as paracetamol, anti-tuberculosis drugs when taken in overdoses, and sometimes when introduced within therapeutic ranges for a long period have been reported to also induce hepatotoxicity [4,5]. Clinically, the injury to the liver generally results in the abnormal variation of serum liver enzymes. Biochemical markers

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include alanine aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, alkaline phosphate, γ -Glutamyl-transpeptidase, serum albumin, and prothrombin time can be traced to reveal abnormalities of hepatic systems or liver function [6].

In the past decades, modern medicine made many advances in the treatment of many diseases. Despite those advances, there are no effective treatments against liver diseases. In addition, many drugs used to treat hepatic diseases induced undesirable side effects and may even be inadequate in curing the damage. Hence, an alternative treatment for these diseases more effective and less toxic is needed [1,7].

Medicinal plants play an essential role in human health. About 80% of the world's population uses traditional medicine as their first care recourse. This traditional medicine is based chiefly on medicinal plants for treating, diagnosing, and preventing diseases. Many scientific studies on medicinal plants have proved that they constitute an alternative solution to many health problems [1]. But on the other hand, many plants are used for medicinal purposes by the population without any scientific proof of their efficacy against diseases and their safety for the patient in terms of their toxicity [8].

Plants from the *Desmodium* genus have been investigated for their pharmacological activities. Most of them exhibited many biological activities *in vitro* and *in vivo*, such as antioxidant, antiviral, immunomodulatory, antidiabetic, anti-inflammatory, cardio-protective and hepatoprotective activities [9]. Among those plants, the *Desmodium repandum* (Vahl) D.C., which is a one-meter high suffrutex plant with trifoliolate leaves in alternate phyllotaxis and orange-red flowers [10] is used in African traditional medicine, either as a single drug or in combination with other plants as an antioxidant, antimicrobial, hemostatic, anti-parasitic and hepatoprotective [11]. Some ethnobotanical studies have reported using *D. repandum* in the treatment of liver diseases, particularly in the South-Kivu province in the East of Democratic Republic of Congo [12] and the Southern part of Rwanda [13]. Despite the use of the plant in traditional medicine, to the best of our knowledge, there is no study up to date that has assessed its hepatoprotective activity in animal models. Thus, the present study aims to assess the hepatoprotective activity of *D. repandum* using a paracetamol induced liver damage animal model in male guineapigs.

2. Material and methods

2.1. Chemicals

Silymarin (Silybin®140) was purchased from Square Herbal and Nutraceuticals Ltd (Bangladesh), Paracetamol tablets from Shalina Ltd (China), Liquid Biochemical Kits for analysis of total bilirubin, AST, and ALT from Cypress Diagnostics (Belgium), and ethanol from Prolabo (Belgium).

2.2. Plant Collection, Processing, and Extraction

Leaves of *D. repandum* were collected from the “Centre de Recherche en Sciences Naturelle de Lwiro (CRSN Lwiro)” in the province of South Kivu in the D.R.Congo during the rainy season and identified in the herbarium A.R. CHRISTIANSEN of the department of Botany of CRSN Lwiro with the number 130. The plant leaves were washed with tap water, dried away from sunlight at room temperature for four weeks, and grounded to powder.

To prepare the plant extract, 250 g of the powder obtained was exhaustively extracted by maceration using ethanol (96%) at room temperature for 72 hours. The resulting extract was filtered through sterile cotton wool and concentrated using a rotary evaporator (PLEDGER) at 40-45 °C. The obtained dried crude extract was kept in labeled glass containers.

2.3. Experimental Animals

Male guinea pigs weighing 175 - 340 g were selected for the experiment. They were kept in individual cages in the light-controlled room (12 h dark/light cycles) in the laboratory of pharmacognosy of the “Université Officielle de Bukavu” for four weeks before the experimentation and they were daily fed with a mixture of edible leaves, mainly *Galisonga ciliata* (Asteraceae) and *Setaria barbata* (Poaceae).

2.4. Preliminary phytochemical screening

The dried extract obtained after exhaustive extraction of *D. repandum* in ethanol was subjected to various qualitative analyses to identify its chemical constituents by using specific reagents commonly used to identify different phytochemicals compounds groups.

2.4.1. Alkaloids

Reagents of Dragendorff and Wagner were used to denote the presence of alkaloids. Few drops of each reagent were added to 2 mL of an aqueous solution of the plant's ethanol extract. For the Dragendorff, the formation of an orange or orange-red precipitate testified to the presence of alkaloids. In contrast, for the Wagner reagent, the shape of a brownish-black precipitate indicated the presence of alkaloids [14,15].

2.4.2. Quinone

Borntraeeger reaction was used to identify quinones. Briefly, 2 mL of concentrate hydrogen chloride was added to 2 ml of the aqueous solution of the extract. The mixture was put in a water bath at 80 °C for 30 minutes then was let to cool down at room temperature. 5 mL of chloroform and 1 ml of diluted ammonia were added to the mixture; the apparition of a red or violet color in the chloroform phase indicated the presence of quinones [15].

2.4.3. Sterols and triterpenes

10 mg of the dried extract was dissolved in 5 mL of acetic anhydride. Thereafter, 0.5 mL of chloroform and then 1 mL of concentrated sulfuric acid was added to the solution (Libermann- Burchards reaction). The formation of a brownish-red ring at the contact zone of the two liquids phases denoted the presence of sterols and triterpenes [16–18].

2.4.4. Coumarins

Approximately 2 mg of dried extract was dissolved in hot water. The solution was divided into two equal volumes: the first one constituted the reference, and the second was made alkaline with 0.5 mL of 10 % ammonia solution. The appearance of an intense fluorescence under ultraviolet light indicated the presence of coumarins and derivatives [16].

2.4.5. Flavonoids

Few drops of concentrated hydrochloric acid were added to 2 mL of an aqueous solution of the extract. After that metallic Mg, and some drops of iso-amyl alcohol were added to the mixture. A red color was developed immediately, which indicated the existence of flavonoids [14,19].

2.4.6. Catechic tannins

To 3 mL of the aqueous solution of the extract, 4 mL of the Stiasny reagent (formaldehyde and concentrated hydrogen chloride in a ratio 2:1) was added. The obtained mixture was placed in a water bath at 80 °C for 30 minutes. The presence of catechin tannins was indicated by the formation of a brown precipitate [18,20].

2.4.7. Gallic tannins

After precipitation of catechic tannins, the supernatant was filtered, and then sodium acetate (5%) and a few drops of iron III chloride (2%) were added to the filtrate. The development of a brownish-green or blue-black color indicated the presence of gallic tannins [15,17,19].

2.4.8. Phenols compounds

1 mL of the aqueous solution of the extract was mixed with 1ml of K₃Fe (C.N.) 6 1% and 1 ml of iron III chloride 2%. The apparition of a blue or green-black color indicated the presence of phenolic compounds [15].

2.4.9. Reducing compounds

1 mL of Fehling solution was added to an aqueous solution of the extract, then the mixture was heated in a water bath at 80 °C. A brick-red precipitate denoted the presence of reducing compounds [16].

2.4.10. Anthocyanins

10 mg of the extract was dissolved in 2 mL, and some drops of concentrated hydrogen chloride were added to the solution. The mixture was placed in a water bath at 80 °C for 5 to 10 minutes under stirring. The apparition of a red color indicated the presence of anthocyanins [16].

2.4.11. Cardiotonic glycosides

Cardio tonic glycosides were identified using the test of Keller-Kiliani, which was done in a test tube plunged in an ice bath. 2 mL of the extract solution was mixed with 1 mL of glacial acetic acid and 1 mL of concentrated sulfuric acid. The

development of a greenish color after adding 2 to 3 drops of iron III chloride 2% confirmed the presence of cardiotoxic glycosides [15].

2.5. Experimental Design

A solution of carboxymethyl cellulose (CMC) 1% was used as a vehicle of all drugs/extracts. The induction of experimental hepatotoxicity in guinea pigs was done by oral administration of paracetamol at a toxic dose of 500 mg/kg body weight [21]. Male guinea pigs were divided into five groups of four animals each and treated as follow:

In the first, which was the control group (C.G.), animals were given the solution of CMC 1% only at the dose of 10 ml/kg body weight. In the second or intoxicated group (I.G.), animals received the solution of CMC 1% (10 ml/kg body weight) first and then paracetamol (500 mg/kg body weight) after one hour. For the third or experimental group 1 (EG1), animals received the extract of *D. repandum* (100 mg/kg body weight) first and then paracetamol (500 mg/kg body weight) after one hour. In the fourth one or experimental group 2 (EG2), animals received the extract of *D. repandum* (200 mg/kg body weight) first and then paracetamol (500 mg/kg body weight) after one hour. In the last group or reference group (R.G.), animals received the reference drug silymarin (100 mg/kg body weight) first and then paracetamol (500 mg/kg body weight) after one hour.

All treatments were given orally by gavage. At the end of the treatment, animals were observed for 24 hours. The animals were then anesthetized with ether-diethyl, and the blood was collected by retro-orbital artery bleeding. Blood samples were centrifuged for 5 minutes at 3000 rotations per minute (rpm) to separate the serum.

2.6. Biochemical Parameters

Liver function biochemical parameters, particularly ALT (alanine transaminase), AST (aspartate transaminase), and total bilirubin levels, were estimated from the serum by using standard kits purchased from Cypress Diagnostics to assess the effect of the plant extract on the damaged liver [22]. The enzyme activity was expressed as IU/L and the total bilirubin in mg/dL.

2.7. Statistical Analysis

The values were represented as the mean \pm standard error mean (SEM) of four animals. A one-way Analysis of variance (ANOVA) test was performed, followed by a Least Significant Difference (LSD) post hoc test for multiple comparisons of means using IBM SPSS Statistics 20 software to determine the level of significance. The value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Preliminary Phytochemical Screening

Table 1 presents the results of the preliminary phytochemical screening of ethanol extract of *D. repandum* leaves. The results revealed the presence of anthocyanin, phenolic compounds, coumarins, flavonoids, quinones, catechic tannins, sterols, and triterpenoids and the absence of alkaloids, cardiotoxic glycosides, reducer sugar, and gallic tannins. In the same genus of *Desmodium*, other plants such as *D. adscendens* and *D. gangeticum* have been investigated for their phytochemicals composition, and the results were comparable to our findings [8,23]. Besides, Domitrovic and Potocnjak discussed currently known molecular mechanisms of the hepatoprotective activity of the 27 most intensively studied phytochemicals. Some of those phytochemicals belong in the groups identified in our studies, such as flavonoids, triterpenes, phenolic compounds, and quinones [24]. Most of the identified phytochemical compounds group in *D. repandum*, which are also in other studied plants, have been reported by many other researchers to possess a wide spectrum of biological activities including hepatoprotective activity [18,23,25–27]. Some pure molecules from the flavonoids group, which showed antioxidant and hepatoprotective activity, had been isolated from *D. adscendens*, the most studied species of the *Desmodium* genus [28,29].

Table 1 Results of the phytochemical screening of the ethanol extract of *D. repandum*

N°	Constituents	Reagents	Observations	Results
1	Alkaloids	Dragendorff	No orange or orange-red precipitate	-
		Wagner	No brownish-black precipitate	-
2	Anthocyanin	Concentrated HCl	Red color	+
3	Phenols compound	K ₃ Fe(CN) ₆ 1% + FeCl ₃ 2%	Blue color	+
4	Coumarins	NH ₄ OH 10%	Intense fluorescence	+
5	Flavonoids	Concentrated HCl + Mg + iso-amylic alcohol	Red color	+
6	Cardiotonic Glycosides	Keller-Kiliani	No greenish color	-
7	Quinone	Borntraëger	Violet color in the CHCl ₃ phase	+
8	Sterols et triterpenes	Libermann-Buchards reaction	A brownish-red ring at the contact zone of the 2 liquids phases	+
9	Reducing compound	Felhing	No brick-red precipitate	-
10	Catechic tannins	Stiasny	Brown precipitate	+
11	Gallic tannins	Na acetate + FeCl ₃ 2%	No brownish-green or blue-black color	-

(+) = presence of constituent, (-) = absence of constituent

3.2. Hepatoprotective Activity

The hepatoprotective potential was investigated in the animal model by observing the change in some biochemical parameters of the liver function, particularly ALT, AST, and bilirubin which are helpful for the detection of hepatic injury [30].

3.2.1. Induction of the liver injury by paracetamol

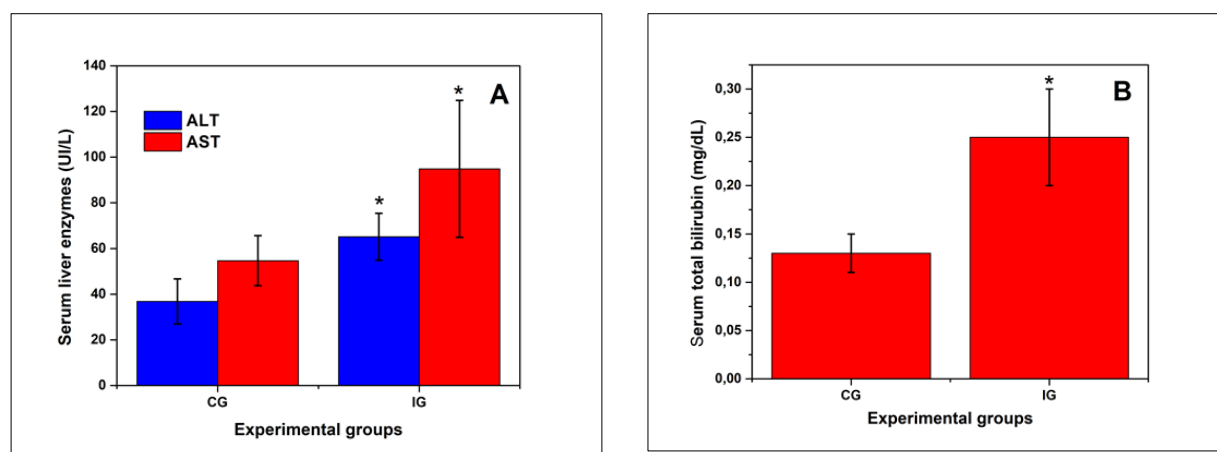


Figure 1 A: Serum enzymes profile, B: Total bilirubin C.G.: Control group, I.G.: intoxicated group, AST: aspartate transaminase, ALT: Alanine transaminase, * $p < 0.05$ vs C.G

In the control group (C.G.), the levels of liver enzymes ALT and AST were 36.79 ± 9.89 IU/L and 54.64 ± 10.97 IU/L (Figure 1A), respectively, and for total bilirubin, it was 0.13 ± 0.02 mg/dL (Figure 1B). These figures are considered as the expected levels of the investigated parameters in this study. For the intoxicated group (I.G.) we observed a significant increase ($p < 0.05$) in total bilirubin 0.25 ± 0.05 mg/dL (Figure 1B), followed by a substantial increase in AST 94.89 ± 30.02 IU/L, and ALT 65.14 ± 10.25 IU/L (Figure 1A) as compared to the control group.

Hepatic injury induced by paracetamol is the most commonly used model for hepatoprotective drug screening. The extent of hepatic damage is assessed by increasing cytoplasmic enzymes like AST and ALT and total bilirubin in blood [8]. Paracetamol is mainly metabolized in the liver by glucuronidation and sulfation [31]. During his metabolism, a part of paracetamol is transformed by the hepatic cytochrome P-450 into N-acetyl-p-benzoquinone imine (NAPQI), a hepatotoxic compound and responsible for paracetamol lever-injury [4]. Thus, in the present study, the increase of total bilirubin and level of transaminases in guinea pigs intoxicated by paracetamol indicates the deterioration of the liver functions and confirms the successful induction of hepatotoxicity in our experimental conditions using paracetamol.

3.2.2. Assessment of the hepatoprotective potential of *D. repandum* ethanolic extract

As depicted in (Figure 2A), significant increases in AST and ALT were observed for EG1 (AST = 86.09 ± 11.95 IU/L and ALT = 62.38 ± 11.67 IU/L) as well as for I.G. (AST = 94.89 ± 30.02 IU/L and ALT = 65.14 ± 10.25 IU/L) compared to the control group with AST and ALT values of 54.64 ± 10.97 IU/L and 36.79 ± 9.89 IU/L, respectively. However, the group EG2 in which animals received a higher dose of 200 mg/kg body weight of the extract showed slightly lower ALT and AST compared to EG1. Furthermore, EG2 also showed the lowest serum bilirubin concentration of 0.15 ± 0.07 mg/dL (Figure 2B) compared to EG1 (0.17 ± 0.08) and R.G. (0.18 ± 0.08) yet not statistically significant when compared with the control group. These results suggested an improved hepatoprotective activity of the ethanolic extract of the plant in a dose-dependent manner. Our results correlate with the one of Jyothi *et al.*, who also found a dose-dependent hepatoprotective activity of *Ixora pavetta* ethanolic extract at the doses of 200 and 400 mg/Kg in an isoniazid-rifampicin induced liver injury in rats [32]. Our study also corroborates with the results of Okokon *et al.*, who find a dose-dependent activity of ethanolic extract of *Homalium letestui* stem in a paracetamol-induced liver injury in rats [33].

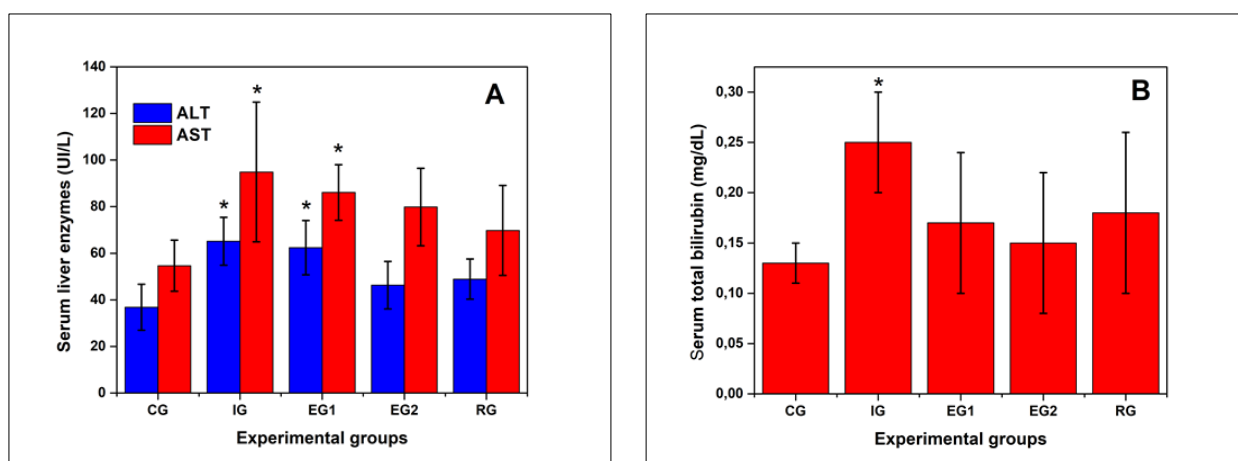


Figure 2 A Serum enzymes profile, B Total bilirubin CG: Control group, IG: intoxicated group, EG1: Experimental group 1, EG2: Experimental groupe 2, RG: Reference groupe, AST: aspartate transaminase

Although I.G. and EG1 showed to have close ALT and AST values (Figure 2A) when comparing all the experimental groups to the C.G., a significant increase in the serum concentration of bilirubin was observed only for the I.G. Noteworthy, the increment in total bilirubin concentration up to 0.17 ± 0.08 mg/dL in the EG1 (Figure 2B) was almost similar to the total serum bilirubin in R.G. (0.18 ± 0.08 mg/dL) which received the reference drug silymarin at the same concentration (100 mg/kg body weight). This indicates a considerable potential hepatoprotective activity of the ethanolic extract of *D. repandum*, taking into consideration that the EG1 received a crude ethanolic extract of the plant, which was proved by phytochemical identification to contain a mixture of compounds of a various group, unlike silymarin which is a mixture of 3 flavonoids molecules (silybin, silydianin, and silychristine) [34].

D. repandum ethanol leaves extract used in this study seems to protect the structural integrity of the hepatocyte membrane. This was evident from the liver protection of *D. repandum* ethanol leaf extract to guinea pigs given paracetamol, which inhibited the rise in blood of transaminases and total bilirubin.

4. Conclusion

The ethanolic extract of *Desmodium repandum* (Vahl) D.C. has anti-hepatotoxic properties because it reduces the harmful effects of paracetamol-induced hepatotoxicity. With a dose-dependent activity, the plant showed a significant reduction in the level of liver biomarkers present in the blood at the dose of 200 mg/kg. Further studies are highly

recommended to isolate the extract's active components and elucidate molecular mechanisms responsible for this hepatoprotection.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest associated with this publication.

Statement of ethical approval

The experiments procedures on animals were performed following the standard ethical guidelines on the protection of animals used for scientific. The protocol was submitted and then approved by Local Ethics Committee of Official University of Bukavu (Protocol no 2017/48).

Author Contributions

Methodology, Investigation, Writing-original draft, Writing-review & editing: J.B.S.; Formal analysis, Methodology, Writing-review & editing: F.M.M; Formal analysis, Writing-review & editing A.M.B.; Conceptualization, Formal analysis, Writing-review & editing: F.K.M; Conceptualization, Supervision, Writing-review & editing: M.J.M. All authors have read and agreed to the published version of the manuscript.

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