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Hepatoprotective effects of leaves aqueous extract of *Rumex bequaertii* De Wild against diclofenac-induced hepatotoxicity

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ABSTRACT

Background: Hepatotoxicity refers to a substance's ability to cause liver tissue damage. This study aimed to evaluate the protective effects of *Rumex bequaertii* De Wild leaf aqueous extract on diclofenac-induced liver damage in Wistar rats. **Materials and Methods:** Thirty-six male rats were divided into six groups of six rats each: a neutral control group received distilled water, a negative control group received diclofenac (25 mg/kg), a positive control group received diclofenac and silymarin (25 mg/kg), two groups received the extract at 50 and 100 mg/kg with diclofenac, and a last group received only the extract at 100 mg/kg. Treatments lasted five days during which silymarin and the extract were administered every day, while diclofenac was given only during the last three days. After treatment, animals were fasted for 12 hours and sacrificed. Blood was collected, and serum was used to measure biochemical parameters. The liver was also collected for oxidative stress evaluation and histological analysis. **Results:** Results showed that diclofenac treatment significantly increased ($p<0.01$; $p<0.001$) ALAT, ASAT, PAL, total bilirubin, total cholesterol, LDL cholesterol and triglycerides while decreasing significantly ($p<0.01$; $p<0.001$) HDL cholesterol. Diclofenac increased also hepatic MDA and NO levels and reduced antioxidant enzymes activity (CAT, SOD, and GSH). Moreover, marked histopathological changes were observed in the negative control group. In contrast, pre-treatment with silymarin and *Rumex bequaertii* extract protected liver tissue from oxidative stress, pathological variations in liver function markers and disruption of its ultrastructure. **Conclusion:** These results suggest that *Rumex bequaertii* De Wild may be exploited as a promising solution to develop an affordable and effective treatment that could thus offer a valuable alternative in combating drug-induced liver damage.

Keywords: *Rumex bequaertii* De Wild, Hepatotoxicity, Diclofenac, Antioxidant.

INTRODUCTION

Diclofenac (DIC) is a non-steroidal anti-inflammatory drug used to treat pain and inflammation. It is derived from phenylacetic acid, which belongs to the aryl carboxylic acid group [1]. Research on diclofenac (DIC) has shown that it can provoke hepatic toxicity by inducing oxidative stress [2]. Indeed, after its absorption, diclofenac is metabolised in the liver to produce metabolites such as 5-OH diclofenac, 4-OH diclofenac, diclofenac-GLuA, and diclofenac-SG. These reactive metabolites induce oxidative stress at the cellular level, leading to cell death through various mechanisms. The reactive metabolites of diclofenac, including 4-OH DIC, 5-OH DIC, DIC-GLuA, and DIC-SG, can disrupt mitochondrial membrane permeability, thereby altering the electron transport chain and resulting in a decrease in oxidative phosphorylation. Consequently, the cell becomes unable to produce ATP, which ultimately leads to its death [3,4]. Furthermore, the permeability of the membrane caused by reactive oxygen species (ROS) triggers the release of pro-apoptotic factors such as cytochrome C, which activates initiator caspase-9. This, in turn, activates the effector caspase-3, which damages genetic material and induces cell death [4]. This hepatic toxicity is characterized by an elevation of the levels of transaminases (ALAT, ASAT), alkaline phosphatase, dyslipidemia (characterized by an elevation of the levels of total cholesterol, LDL cholesterol, triglycerides, and a decrease in the level of HDL cholesterol) and a decrease in the activity of antioxidants (GSH, CAT, SOD) [5,6]. This hepatotoxicity leads to the development of pathologies such as jaundice, hepatic fibrosis, cholestasis, and non-alcoholic fatty liver disease [7].

Globally, drug-induced hepatitis affects approximately 20 people per 100,000 [8] and is a major cause of liver failure, sometimes fatal if left untreated [9,10]. Furthermore, they are responsible for the withdrawal of numerous drugs from the market [11]. The primary treatment for advanced liver failure due to medications is liver transplantation [12]. Non-surgical treatments include non-specific medications such as silymarin and corticosteroids, as well as the use of N-acetylcysteine as an antidote, for example, in

cases of paracetamol-induced hepatitis [12]. However, these treatments can lead to side effects such as diarrhea, vomiting, skin rashes, headaches, pain, and muscle cramps. In contrast, plants are generally well-tolerated by the body, and certain plants possess compounds capable of protecting the liver against medication-induced damage. Also known as spinach-sorrel or perpetual spinach, *Rumex bequaertii* is a perennial, herbaceous plant of Polygonaceae family growing up to two meters. It is used in traditional medicine in Cameroon to treat rheumatism, malaria, bacterial infections and amoebic dysentery. Investigation into this plant has shown that it possesses laxative, diuretic, antipyretic, antioxidant, wound-healing, anti-inflammatory and anti-ulcer properties [13,14]. Given the lack of accessible treatments with fewer side effects against drug-induced hepatotoxicity, this plant could be used as an alternative in the treatment of drug-induced hepatitis. In this study, we evaluate the protective effects of *Rumex bequaertii* De Wild leaf aqueous extract on diclofenac-induced liver damage in Wistar rats.

MATERIALS AND METHODS

Chemicals

Diclofenac sodium (75mg/3mg) and silymarin (140mg) were purchased from a pharmacy.

Experimental Animals

The animal material consisted of male Wistar rats bred at the animal house of the University of Dschang (Cameroon) in the unit of animal physiology and pharmacology research under a natural cycle (12/12 hours day/night). They were approximately 4 months old and weighed around 250g. These animals had free access to water and standard food. All tests in this study were conducted according to the ethical standards described by law number 2010/63/EU of the European Parliament and Council of September 2010 for the protection of animals used for scientific research.

Harvesting and authentication of *Rumex bequaertii* De Wild

The leaves of *Rumex bequaertii* De Wild were harvested in Cameroon, in the town of Dschang, in March 2024. They were authenticated in Yaoundé at the National Herbarium of Cameroon under the number 44158HNC, by comparison with the specimen 8779HNC of AJM Leeuwenberg dated 27/09/1971. These leaves were used for the preparation of the aqueous extract.

Preparation of the aqueous extract of *Rumex bequaertii* De Wild

The harvested leaves of *Rumex bequaertii* De Wild were dried in the shade and powdered for extract preparation. For this purpose, 500g of powder was introduced into 5L of boiling distilled water for 20 minutes. The resulting decoction was left to cool at room temperature and filtered with cotton and, then Whatman paper number 4. The filtrate obtained was dried at 40°C for 72 hours, yielding 116g of dry extract used for animal treatment.

Phytochemical screening

Qualitative screening

The determination of the classes of compounds present in this extract was carried out according to the standard methods described by Harborne (1973) [15]. In this test, the presence of compounds such as alkaloids, phenols, flavonoids, steroids, triterpenoids, tannins, saponins, anthocyanins, and anthraquinones was investigated.

- **Alkaloids assay (Meyer's test)**

Zero point zero one gram (0.01 g) of extract was placed in a test tube with 3 ml of 50% (v/v) hydrochloric acid aqueous solution. The mixture was treated with 3 drops of Meyer's reagent. The formation of a white or yellowish precipitate indicated the presence of alkaloids.

- **Phenol Assay**

Zero point zero one-gram (0.01 g) of extract was dissolved in 3 ml of ethanol, then the mixture received 3 drops of 10% (v/v) Iron (III) chloride. The appearance of a blue-violet or greenish color indicated the presence of phenols.

- **Flavonoid Assay (Shinoda Test)**

Zero point zero one gram (0.01 g) of extract was dissolved in 3 ml of methanol. The mixture was treated with 0.05 g of magnesium shavings and 3 drops of concentrated HCl. The presence of flavonoids was detected by colorations ranging from orange to red or pink.

- **Assay for Triterpenes and Steroids: Liebermann-Burchard Test**

Zero point zero one gram (0.01 g) of extract was dissolved in 3 ml of chloroform, then 3 ml of acetic anhydride were added and the mixture was cooled in ice for 3 minutes. Finally, one drop of concentrated sulfuric acid was added. The presence of triterpenes was confirmed by the appearance of a reddish-violet color, and steroids by the successive appearance of blue, green, red, or orange colors.

- **Tannins Assay**

Zero point zero one gram (0.01 g) of extract was boiled for 5 minutes in a tube containing 5 ml of water. After cooling, the mixture received 5 ml of 2% (w/v) sodium chloride (NaCl) and 5 ml of 1% (w/v) gelatin. The appearance of a precipitate confirmed the presence of tannins.

- **Saponins assay**

Zero point zero one gram (0.01 g) of extract was dissolved in 5 ml of distilled water, then boiled for 5 minutes. After cooling, the contents of the 15x160 mm test tube were vigorously shaken for 30 seconds, then left to stand. The appearance of persistent foam with a height of more than 1 cm characterized the presence of saponins.

- **Anthocyanins assay**

Zero point zero one gram (0.01 g) of extract was boiled in the presence of 5 ml of 1% (v/v) hydrochloric acid (HCl) aqueous solution. The color change was noted. The presence of anthocyanins was indicated by an orange color.

- **Anthraquinones assay**

Zero point zero one gram (0.01 g) of extract was dissolved in a mixture of 4 ml of ether-chloroform (1:1 v/v). The resulting solution was treated with 4 ml of 10% (w/v) sodium hydroxide (NaOH). The quinones were identified by the presence of a red coloration.

Quantitative screening

Total phenolic, flavonoids, and tannins content were also quantified in this study.

- **Total phenolic content assay**

The quantification of total phenols was carried out using the method described by Ramde-Tiendrebeogo *et al.* (2012) [16]. For this analysis, a reaction mixture was prepared by mixing 20 µL of the aqueous extract of *Rumex bequaertii* leaves (at a concentration of 2 mg/mL), 100 µL of Folin-Ciocalteu reagent (diluted 10-fold in water), and 80 µL of 20% sodium carbonate solution. The mixture was shaken and incubated in a water bath at 20°C for 30 minutes, then the absorbance was measured using a spectrophotometer at 765 nm. Blank tubes

containing distilled water were used as references for correction. A calibration curve was established using gallic acid as the standard. The results were expressed in milligrams of gallic acid equivalents per gram of extract (mg AE/g).

- **Total flavonoid content assay**

Flavonoids were quantified according to the aluminum chloride colorimetric method described by Chang *et al.* (2002) [17]. A volume of 100 µL of the aqueous extract of *Rumex bequaertii* leaves (at a concentration of 2 mg/mL) was mixed with 50 µL of 1.2% aluminum chloride and then with 50 µL of 120 mM potassium acetate. The mixture was incubated at room temperature for 30 minutes, then the absorbance was measured at 415 nm using a spectrophotometer. The total flavonoid content was calculated based on the quercetin calibration curve. The results were expressed in milligrams of quercetin equivalents per gram of extract (mg QE/g)."

- **Total tannins content assay**

Tannins were quantified according to the Folin-Ciocalteu method described by Govindappa *et al.* (2011) [18]. For this assay, a reaction mixture was prepared by combining 100 µL of the aqueous extract of *Rumex bequaertii* leaves (at a concentration of 2 mg/mL), 500 µL of Folin-Ciocalteu reagent (diluted 10-fold in water), 1000 µL of 35% sodium carbonate solution, and 8.4 mL of distilled water. The mixture was shaken and incubated at room temperature for 30 minutes, then the absorbance was measured using a spectrophotometer at 700 nm. A tube containing distilled water was used as a blank for correction. A calibration curve was constructed using tannic acid as the standard. The result was expressed in milligrams of tannic acid equivalents per gram of extract (mg TAE/g).

Hepatoprotective effects of *Rumex bequaertii* De Wild

Distribution and treatment of animals

The experimental animals were individually labeled, and divided into six groups of six rats each in plastic cages and treated as follows:

- Group 1: Neutral control, received distilled water (10 ml/kg p.o) for the first two days. From the third day, NaCl (0.9%) was administered at a dose of 1 mg/kg intraperitoneally, one hour after distilled water administration until the fifth day;

- Group 2: Negative control, received only distilled water (10 ml/kg p.o) for the first two days. From the third day, diclofenac sodium was administered at a dose of 25 mg/kg intraperitoneally, one hour after distilled water administration until the fifth day;

- Group 3: Positive control, received only silymarin (25 mg/kg p.o) for the first two days. From the third day, diclofenac sodium was administered at a dose of 25 mg/kg intraperitoneally, one hour after silymarin administration until the fifth day;

- Group 4: Received only the aqueous extract of *R. bequaertii* De Wild at a dose of 50 mg/kg (p.o) for the first two days. From the third day, diclofenac sodium was administered at a dose of 25 mg/kg intraperitoneally, one hour after extract administration until the fifth day;

- Group 5: Received only the aqueous extract of *R. bequaertii* De Wild (100 mg/kg p.o) for the first two days, then from the third day, in addition to the extract, one hour later, rats in this group received diclofenac sodium at a dose of 25 mg/Kg intraperitoneally until the fifth day;

- Group 6: Received only the aqueous extract of *R. bequaertii* De Wild (100 mg/kg p.o) for the first two days, then from the third day, in addition to the extract, one hour later, NaCl 0.9% (1 ml/Kg i.p) was administered until the fifth day;

On the last day of treatment (5th day), the animals were fasted for 12 hours, then anesthetized with diazepam (10 mg/kg) and ketamine (50 mg/kg). After this, blood was collected by catheterization of the abdominal artery and introduced into dry test tubes, then centrifuged at 3000 rpm for 15 minutes. The serum collected after centrifugation was introduced into Eppendorf tubes and stored at -20°C for biochemical analyses. The liver was removed, cleared of fat and drained. One part was used for preparing homogenates for measuring oxidative stress parameters, and the other part was introduced into 10% formalin for histological analysis.

Statistical analysis

The analysis of results was performed using GraphPad Prism software version 8.4.2. The results were expressed as mean ± standard error of the mean (M±SEM). A one-way ANOVA test followed by Tukey's post-test (Multiple comparison test) was used. The probability threshold $p < 0.05$ was considered significant.

RESULTS

Qualitative composition of *Rumex bequaertii* De Wild

These results show that the aqueous extract of *Rumex bequaertii* De Wild leaves contains nine classes of chemical compounds summarized in Table 1.

Quantitative composition of *Rumex bequaertii* De Wild

The aqueous extract of *Rumex bequaertii* De Wild leaves contains a range of phenolic compounds (8.4 ± 0.23), with flavonoids (5.156 ± 0.35) being the most abundant, followed by tannins (2.05 ± 0.24), as shown in Table 2.

Effects of the aqueous extract of *Rumex bequaertii* De Wild on liver function markers

The results presented in Table 3 show the effects of treatments on biochemical parameters. It appears that the administration of diclofenac alone significantly increases ($p<0.01$; $p<0.001$) the levels of ALAT, ASAT, PAL, total bilirubin, total cholesterol, LDL cholesterol, and triglycerides, but decreases significantly ($p<0.01$) the level of HDL cholesterol compared to the neutral control. In contrast, pre-treatment of rats with silymarin and different doses of extracts significantly ($p<0.05$; $p<0.01$; $p<0.001$) prevented the increase in these parameters compared to the negative control.

Antioxidant effects of the aqueous extract of Rumex bequaertii De Wild leaves

The results presented in Table 4 reveal the impact of treatments on oxidative stress status. It appears that diclofenac administration led to a significant ($p<0.001$; $p<0.001$) decrease in GSH, SOD, and CAT activity, but significantly ($p<0.001$) increased hepatic NO and MDA levels compared to the neutral control. Pre-treatment with silymarin and different doses of extract significantly ($p<0.05$; $p<0.01$; $p<0.001$) prevented these variations.

Effects of the extract on liver tissue architecture

Histological analysis revealed that in the neutral control group, the microarchitecture of the hepatic parenchyma is normal, with a distinct structure comprising a portal space (portal vein, hepatic artery, bile canaliculus, hepatocyte, and sinusoidal capillary). In contrast, the negative control group shows marked histopathological alterations characterized by the presence of lysed hepatocytes. The groups receiving different doses of the extract, as well as the positive control group, demonstrate improved hepatic microstructure compared to the negative control group, as illustrated in the Figure 1.

Table 1: Compounds present in the aqueous extract of *Rumex bequaertii* De Wild

Classes of compounds								
Alkaloids	**Phenols**	**Flavonoids**	**Sterols**	**Triterpenoids**	**Tannins**	**Saponins**	**Anthocyanins**	**Anthraquinones**
+	+	+	+	+	+	+	+	+

+: indicates the presence of the different classes of chemical compounds in the aqueous extract of *R. bequaertii* leaves

Table 2: Quantification of phenol in the aqueous extract of *Rumex bequaertii* De Wild leaves

	TPC (mg GAE/g of extract)	TFC (mg QE/g of extract)	TTC (mg TAE/g of extract)
***Rumex bequaertii* De Wild**	8.4 ± 0.23	5.156 ± 0.35	2.05 ± 0.24
Butylated hydroxytoluene	435.21 ± 0.51	73.29 ± 0.33	62.42 ± 0.44

TPC: Total Phenol Content; TFC: Total Flavonoid Content; TTT: Total Tannin Content; EAG: Gallic Acid Equivalents; QE: Quercetin Equivalents; TAE: Tannic Acid Equivalents

Table 3: Effects of the aqueous extract of *Rumex bequaertii* De Wild on biochemical parameters

Parameters	Neutral control	Negative control	Positive control	AE50 + DIC	AE100 + DIC	AE 100
ALT (UI/L)	14.45 ± 0.70	27.24 ± 1.34***	15.10 ± 1.78^{c}	16.34 ± 3.04 ^{c}	20.17 ± 2.91 ^{c}	13.12 ± 1.91
AST (UI/L)	32.08 ± 0.63	79.84 ± 2.25***	62.71 ± 1.81^{a}	59.94 ± 4.40^{b}	55.48 ± 3.74 ^{c}	48.27 ± 3.88
ALP (UI/L)	433.00 ± 33.64	787.10 ± 25.68***	224.50 ± 56.66 ^{c}	487.60 ± 64.33 ^{b}	552.90 ± 36.76 ^{a}	418.80 ± 43.59
Total bil (µmol/L)	8.06 ± 0.13	16.70 ± 0.65***	9.64 ± 0.69 ^{c}	11.41 ± 0.65^{c}	10.99 ± 0.37 ^{c}	10.03 ± 0.93
Total-Chol (mmol/L)	3.62 ± 0.17	4.89 ± 0.14**	3.08 ± 0.26 ^{c}	2.85 ± 0.11 ^{c}	3.23 ± 0.13 ^{c}	3.10 ± 0.30
HDL-Chol (mmol/L)	0.76 ± 0.08	0.44 ± 0.02**	0.51 ± 0.03	0.47 ± 0.08	0.54 ± 0.03	0.83 ± 0.06
LDL-Chol (mmol/L)	2.42 ± 0.16	3.84 ± 0.36**	1.91 ± 0.26^{c}	1.79 ± 0.10 ^{c}	2.19 ± 0.13 ^{c}	2.99 ± 0.30
TGL (mmol/L)	1.80 ± 0.04	3.86 ± 0.16***	2.31 ± 0.31 ^{c}	2.147 ± 0.16 ^{c}	2.166 ± 0.06 ^{c}	1.39 ± 0.10

The results were expressed as mean ± standard error of the mean (SEM). N=6; **p<0.01, and ***p<0.001 compared to the neutral control; ^{a}p<0.05, ^{b}p<0.01 and ^{c}p<0.001 compared to the negative control. ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Phosphatase Alkaline; Total bil: Total bilirubin; Total-Chol: total cholesterol; HDL-Chol: High-Density Lipoprotein cholesterol; LDL-Chol: Low-Density Lipoprotein cholesterol; TGL: Triglyceride

Table 4: Effects of *Rumex bequaertii* De Wild aqueous extract on oxidative stress

Parameters	Neutral control	Negative control	Positive control	AE50 + DIC	AE100 + DIC	AE 100
GSH (µmol/g tissue)	150.70 ± 4.52	116.10 ± 1.54***	196.90 ±3.23^{c}	137.40 ± 2.78^{b}	135.90 ± 3.02^{b}	169.30 ± 4.09^{c}
SOD (mUI/g de tissu)	21.87 ± 0.44	6.45 ± 0.63***	11.19 ± 0.704^{a}	12.96 ± 1.41^{c}	14.37 ± 1.33^{c}	11.69 ± 0.846^{b}
CAT (H_2O_2/mg protein)	0.13 ± 0.02	0.03 ± 0.00***	0.12 ± 0,02^{b}	0.11 ± 0,00^{b}	0.13 ± 0,01^{c}	0.16 ± 0,00^{c}
MDA (µmol/g tissue)	1.24 ± 0.12	4.16 ± 0.32***	1.44 ± 0.10^{c}	1.91 ± 0.25 ^{c}	1.45 ± 0.15 ^{c}	1.78 ± 0.20 ^{c}
NO (µmol/g tissue)	16.86 ± 0.41	41.60 ± 1.94***	22.33 ± 1.24 ^{c}	12.51 ± 1.59 ^{c}	23.85 ± 4.33 ^{c}	32.82 ± 1.56

The results were expressed as mean ± standard error of the mean (SEM). N=6; ***p<0,001 compared to the neutral control; ^{a}p<0,05, ^{b}p<0,01 and ^{c}p<0,001 compared to the negative control. GSH: Glutathione; SOD: Superoxide Dismutase; CAT: Catalase; NO: Nitric Oxide; MDA: Malondialdehyde

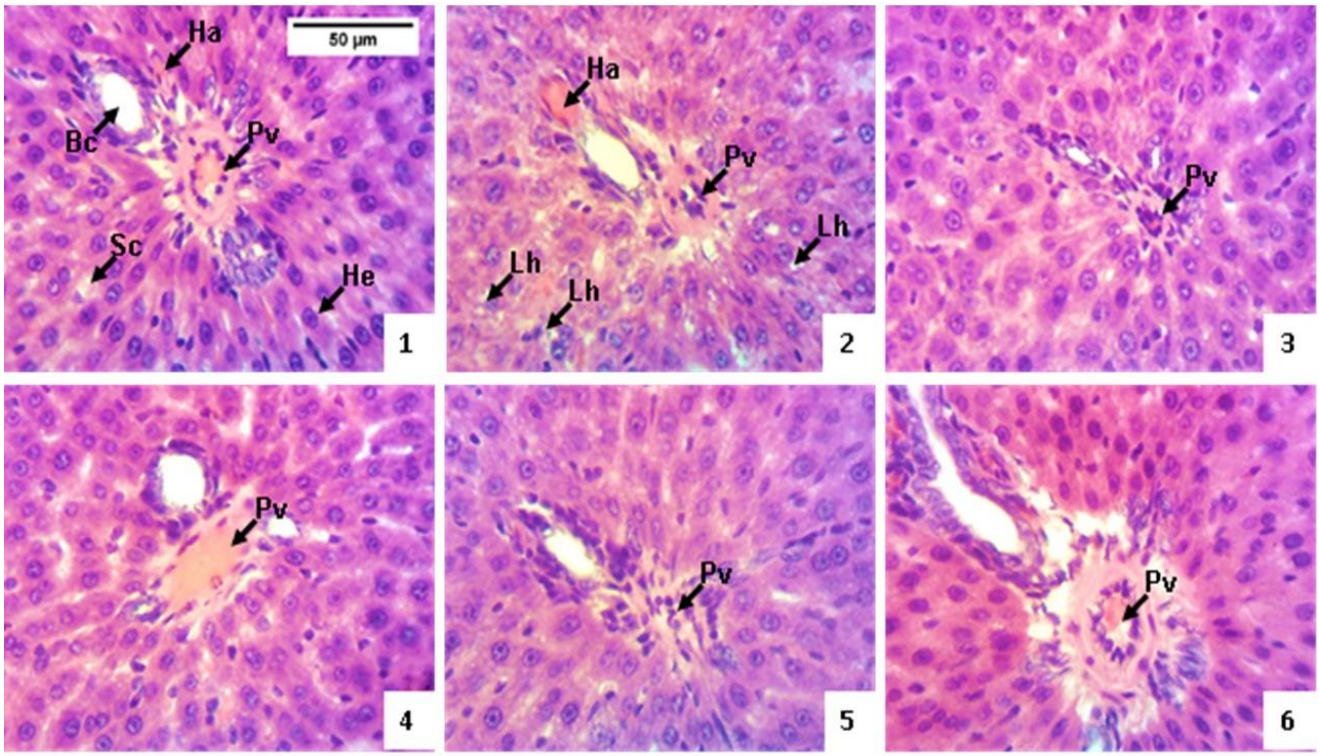

Figure 1: Liver microphotographs at X250

1 = Neutral control; **2** = Negative control; **3** = Positive control; **4, 5** = Groups receiving the extract at respective doses of 50 and 100 mg/kg; **6** = Satellite; **Pv** = Hepatic portal vein; **He** = Hepatocyte; **Sc** = Sinusoidal capillary; **Bc** = Bile canaliculus; **Ha** = Hepatic artery; **Lh** = Lysed hepatocytes

DISCUSSION

Since its market introduction, diclofenac has remained one of the most popular and preferred non-steroidal anti-inflammatory drugs in many households for the treatment of pain and inflammation. This preference is due to its efficacy, affordability, and availability over the counter. Diclofenac belongs to the aryl carboxylic group, derived from phenylacetic acid [19]. Despite its beneficial effects, several studies have highlighted its toxicity in users and animal laboratory models [20]. This toxicity is particularly concerning for certain organs such as the kidneys, stomach, and liver [20]. The primary objective of this study was to evaluate the hepatoprotective effects of the aqueous extract of *Rumex bequaertii* De Wild leaves on various aspects of diclofenac-induced hepatotoxicity. Conventional treatments for drug-induced hepatitis are often associated with several side effects, justifying the growing interest in phytotherapy, known for its good tolerance.

Hepatotoxicity manifests as an increase in liver enzyme levels, such as transaminases (ALAT and ASAT) and PAL, which are markers of liver tissue integrity [21] It is also characterized by a decrease in antioxidant compounds like GSH, CAT, and SOD, as well as an increase in lipid peroxidation in hepatocytes, indicated by elevated MDA levels. The toxicity of diclofenac is primarily associated with oxidative stress, leading to inflammation that exacerbates liver damage [20].

The transaminases (ALAT, ASAT) and PAL are enzymes primarily synthesized by the liver and play a crucial role in evaluating the integrity and function of hepatocytes. When hepatocytes are lysed, these enzymes are released in large quantities into the bloodstream [19]. The administration of diclofenac sodium at a dose of 25 mg/kg resulted in a significant increase in the levels of these enzymes, clearly indicating its hepatotoxicity. These results are similar to those of Esmailzadeh *et al.* (2020) [22], who showed an increase in ALAT and ASAT levels after diclofenac administration. This elevation is attributed to the lesions (or cytolysis) suffered by the liver tissue, caused by oxidative stress and increased inflammation in the liver. These cytolyses are observed in histological sections. Pre-treatment with the extract significantly prevented the increase in these enzymes. This preventive effect is likely linked to the phenolic compounds (flavonoids and tannins) present in this plant. These chemical compounds have a strong antioxidant power capable of boosting the body's antioxidant system and providing electrons necessary to stabilize reactive oxygen species [13].

Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, in favor of the former. When their production increases, the body mobilizes various defense systems to counteract their oxidizing power. However, in cases of overproduction, these defenses can become ineffective, allowing ROS to cause tissue damage [23]. SOD, CAT, and GSH are the main defense systems of the body against oxidative stress. In case of increased stress, their levels may decrease.

Malondialdehyde (MDA) is a final product of lipid peroxidation. When lipids are attacked by reactive oxygen species (ROS), mainly the hydroxyl radical, they can remove hydrogens from the double bonds of fatty acids, leading to the formation of conjugated diene radicals, which are then oxidized to hydroxyl radicals, a process known as lipid peroxidation [2] The resulting peroxyl radical can react with other fatty acids to generate new conjugated radicals. This process can be inhibited by glutathione peroxidase, however, in cases of increased stress, this system becomes ineffective, and these radicals are oxidized to form aldehydes, including malondialdehyde [2,24]. Diclofenac administration led to a significant increase in its level, indicating that diclofenac induces lipid peroxidation of the biomembranes of hepatocytes. Pre-treatment with the extract at all doses significantly prevented this increase, which would translate to the anti-peroxidant effect of this plant.

Reduced glutathione (GSH) is the main antioxidant present in the body's cells and plays a crucial role in protecting against damage caused by stress. To fulfill this protective function, GSH acts by neutralizing free radicals by providing them with an electron, which reduces their reactivity. Additionally, it indirectly contributes to

protection by regenerating other antioxidants like vitamins C and E, providing them with the necessary electrons for their proper functioning [25]. When stress levels increase, the demand for GSH also increases, but the body may not be able to produce enough GSH to meet this increased demand, leading to a decrease in its levels in cells. Diclofenac administration induced a significant decrease in its levels, supporting the hypothesis that diclofenac's toxic effect involves oxidative stress. These results are consistent with those of Esmaeilzadeh *et al.* (2020) [22]. Similarly, the decrease in SOD and CAT levels was observed with diclofenac administration. When these defense mechanisms against stress become ineffective, it also affects the liver's ability to perform its functions, including its ability to metabolize various substances in the body. Pre-treatment with the extract at all doses significantly prevented the decrease in these antioxidants in liver tissue. This preventive effect would be linked to the plant's ability to boost the body's antioxidant defense system.

During drug-induced hepatotoxicity, NO production is often stimulated by the activation of Kupffer macrophages in the liver in response to cellular damage. NO is synthesized from L-arginine by the enzyme nitric oxide synthase (NOS), mainly in its inducible form (iNOS) in situations of oxidative stress Husam, marangoni [26,27]. This increase in NO contributes to cytotoxicity by promoting lipid peroxidation. In this present study, diclofenac caused a significant increase in NO levels in the negative control group. These results are in agreement with those of Nouri *et al.* (2019) [20], which show the role of NO in diclofenac-induced hepatotoxicity. However, pre-treatment of rats with the aqueous extract of *Rumex bequaertii* De Wild leaves inhibited this increase in NO. This inhibition would be linked to *Rumex bequaertii*'s ability to reduce iNOS activation and neutralize free radicals. These results are consistent with the observations of Duarte *et al.* (2014) [28], which show the modulatory effects of flavonoids on NO.

The liver is the only organ responsible for bilirubin metabolism [21]. After its production, bilirubin is released into the blood where it binds to albumin, then it is conjugated in the liver by uridine diphosphate glucuronosyltransferase to form conjugated bilirubin. This is then excreted into the bile by the bile canaliculi for elimination. When the liver is damaged, its ability to metabolize bilirubin is reduced, leading to an accumulation of unconjugated bilirubin in the bloodstream and an increase in total bilirubin levels [21]. Diclofenac administration caused an increase in total bilirubin levels in the blood, which again highlights the hepatotoxic nature of this NSAID. Similar results were observed by Nouri *et al.* (2019) [20] Pre-treatment with the extract at all doses significantly prevented the increase in bilirubin observed with diclofenac. This effect could be explained by the extract's ability to stimulate liver cells to conjugate bilirubin and excrete it into the bile.

The liver also plays a crucial role in fatty acid metabolism [21]. When it is damaged, its ability to metabolize fatty acids is compromised, which can result in a decrease in HDL cholesterol levels and an increase in total cholesterol, LDL cholesterol, and triglyceride levels [20]. Diclofenac sodium administration led to a decrease in HDL cholesterol levels and an increase in triglyceride, total cholesterol, and LDL cholesterol levels. These results corroborate with those observed by Jia *et al.* (2023) [29] and Nouri *et al.* (2019) [20] and once again demonstrate the hepatotoxicity of diclofenac. Administration of silymarin and the aqueous extract of *Rumex bequaertii* De Wild leaves at all doses prevented these changes, except for the variation observed with HDL cholesterol levels.

The observed results in this study, could be due to the presence of various important medicinal compounds underlined during the phytochemical analysis, such as phenols, flavonoids, sterols, alkaloids, tannins, triterpenoids, anthocyanin, anthraquinones, saponines, which are plants components that have been researched primarily for their potential activity against oxidative and inflammatory injuries induced-hepatotoxicity [30].

CONCLUSION

At the end of this study, which aimed to evaluate the hepatoprotective effects of the aqueous extract of *Rumex bequaertii* De Wild leaves on liver damage induced by diclofenac sodium in Wistar rats, it appears that this aqueous extract prevented pathological alterations in biochemical parameters and oxidative stress. Furthermore, it protected the liver tissue against cytolysis and disorganization of its ultrastructure.

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Ethical considerations

All animals were handled in accordance with the guidelines of the Cameroon National Veterinary Laboratory (No. 003/19 CCS/MINEPIA/RD-NW/DDME/SSV). The experimental protocol was reviewed and approved by the Scientific Committee of the Department of Animal Biology, University of Dschang, Dschang, Cameroon.

Conflict of interest

The authors declared no conflict of interest.

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