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HEPATOPROTECTIVE EFFECTS OF EXTRACTS AND SOLVENT FRACTIONS FROM *MORINDA MORINDOIDES* (BAKER) MILNE-REDHEAD (RUBIACEAE) LEAVES IN EXPERIMENTAL SWISS ALBINO MICE

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ABSTRACT

Morinda morindoides leaves aqueous extract and its chloroform, ethyl acetate and *n*-butanol fractions and residual aqueous phase, as well its methanol 80% extract and crude polysaccharides, were evaluated for their potential hepatoprotective activity against carbon tetrachloride-induced liver toxicity. The intraperitoneal injection of CCl_4 in Swiss albino mice induced significant increases of hepatic enzymes, such as alanine transaminase (ALT), aspartate transferase and alkaline phosphatase (ALP), superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) and other biochemical parameters, such as albumin, total proteins, and decreased urea a uric acid, decreased hematological parameters such as fasting blood glucose (FBG), total cholesterol (TC), low density lipoproteins (LDL) and increased triglyceride (TG) and high density lipoproteins (HDL) respectively and fasting blood glucose (FBG). Inversely, treatment with aqueous extract at high oral administered dose of 400 mg/kg bodyweight, induced significant decrease of biochemical enzymes and triglycerides, bilirubin, creatinine and low density lipoproteins (LDL), urea and uric acid, and increases total proteins, total cholesterol (TC) and high density lipoproteins (HDL) and platelets. Chloroform, ethylacetate, *n*-butanol and residual phase solvent fractions produced also similar effects as aqueous extract on all selected biochemical parameters and products. The hepatoprotective activity of aqueous extract from *Morinda morindoides* leaves used at 400 mg/kg bodyweight was comparable to Silymarin used as reference product used at 100 mg/kg bodyweight. Obvious or seeing the effects of aqueous and solvent fractions on biochemical parameters and products, it can be assumed, these samples and Silymarin reference product, possessed interesting and appreciable hepatoprotective effects comparable to Silymarin and can be used as an adjuvant in the management of acute injuries and treatment of drug-related hepatic injuries and degenerative diseases.

KEYSWORDS: *Morinda morindoides*, Rubiaceae, leaves, crude polysaccharides, liver, hepatoprotective activity, *in vivo* study.

1. INTRODUCTION

Hepatotoxicity can be defined as the effect of an agent on the liver resulting in a deviation from its normal functions, morphology, including chemical substances among which certain drugs, or microbial-driven hepatotoxicity. Hepatotoxicity or liver damage is characterized by metabolic dysfunction and histopathological patterns such as granuloma, cholestasis, hepatitis, neoplasms, steatosis and zonal necrosis representing 5% of all liver injuries. As a consequence, over a million casualties are reported annually due to the indirect result of liver disfigurement

or hepatocellular carcinoma (Boro et al., 2022). Some agents can lead to necrosis, steatosis, cirrhosis, liver carcinoma, etc., while others cause jaundice with little or no overt injuries to the hepatic parenchyma and produce degenerative and vascular lesions (Klinchi and Katiyar, 2017).

The liver is one of the most complex organs in the human body and plays a key role in the metabolism, detoxification and excretion of xenobiotics. Common causes of liver injuries are viral infections, xenobiotics, toxic chemicals, excessive drug use, environmental pollutants, chronic alcohol abuse and certain drugs, such as paracetamol, rifampicin, and chemotherapeutic drugs, as well as natural toxins, such as peptides of *Amanta phalloides*, or pyrrolizidine alkaloids (Ubhenin et al., 2016; Singh et al., 2011, Naveen et al., 2016; Arun and Eswar, 2013,. Samaresh et al., 2013; Klinchi and Katiyar, 2017). Many drugs, toxins and herbal medicines have been reported to cause liver injuries and drugs account for 20-40% of hepatic failure (Geresu et al. 2022).

Furthermore, the liver produces bile, which is crucial for the proper digestion of fats in the gastrointestinal tract. Liver injury or dysfunction is recognized as a serious global health problem. Clinically available synthetic drugs for the treatment of liver diseases, such as interferon and corticosteroids are expensive, particularly for patients living in developing countries, and can have significant side effects (Yang et al., 2018). However, the drugs used to treat liver diseases are unsatisfactory, because they do not offer complete protection to the organ and can exert serious long-term undesirable effects (Boro et al., 2022).

Medicinal plants offer a promising, cost-effective, and low-toxicity alternative to modern, limited pharmacological treatments for liver disorders, with over 160 phytochemicals from more than 100 medicinal plant species demonstrating hepatoprotective activity. These plants primarily work via antioxidant and free radical scavenging, and anti-inflammatory mechanisms, stabilizing liver cells against chemically induced damages.

Herbal drugs are more broadly used than allopathic drugs as hepatoprotective drugs because they are less expensive, higher cultural acceptability, higher compatibility, with the human body and minimum facet results. These herbal drugs have shown the potential to hold the normal useful statues of the liver with or without fever facet results.

Therefore, a large range of plant species and formulations have been claimed to have hepatoprotective activities so the improvement of plant based totally hepatoprotecting drugs has been given significance within the global market place (Klinchi and Katiyar, 2017).

Liver diseases can be classified as acute or chronic and include a.o. hepatitis and cirrhosis). They constitute a worldwide problem causing the death of about 25,000 people annually due to liver cirrhosis caused by hepatitis and lesions due to the injuries induced by drugs such as paracetamol (Medjahed et al., 2016).

Given the significant number of people suffering from liver diseases and the drawbacks in currently available therapies, there is still a need for novel hepatoprotective therapies. One potential source of these could be traditional medicine (Geresu et al., 2022), including medicinal plants. *Morinda morindoides* is a medicinal plant currently used in many African countries, and particularly in the Democratic Republic of Congo. In this country, the current preparation used in traditional medicine is an aqueous decoction of the leaves. This decoction is used to orally treat various diseases like, diabetes (mainly type 2), rheumatism, gale, wounds, fever including fever caused by malaria, constipation pains, urogenital infections, gastralgia, general tiredness, amoebiasis, and is used as a tonic, stimulant of appetite and vermifuge.. (Kambu, 1990).

Several studies were previously conducted and confirmed that the aqueous extract (decoction) and its soluble fractions were endowed with different biological activities, including complement-inhibiting activity (Cimanga et al., 1993, 2003), *in vitro* anticomplementary activity (Cimanga et al., 1995, 1997), antibacterial and antifungal (Cimanga et al., 1991, 1997, 1997)), antioxidant (Cos et al., 1998, Cimanga et al., 1999, 2021), antiplasmodial in *vitro* and *in vivo* (Tona et al. 2001, Cimanga et al., 2008), anti-amoebic *in vitro* (Cimanga et al., 2006a, b), spasmolytic on isolated ileum from guinea-pig (Cimanga et al., 2010), antidiarrheal *in vivo* (Mbamu et al, 2019), antidiabetic *in vivo* (Cimanga et al., 2021b) and anti-inflammatory and analgesic *in vivo* activity (Cimanga et al., 2025).

With regard to the chemical composition, some flavonoids, anthraquinones, (Cimanga at al., 1995, 1997) and iridoids (Cimanga et al., 2013) were previously isolated from *M. morindoides* leaves. Furthermore, a positive test upon treatment with Dragendorff reagent could indicate the presence of alkaloids. However, the possibility of a false positive result (related to the presence of iridoids) should be taken into account (Cimanga et al., 2013). and to the best of our knowledge, until now, there are no reports in the literature concerning the isolation of alkaloids from *M. morindoides*.

However, only limited informations are available about the potential beneficial effects of *M. morinodoides* in hepatic pathological conditions. Therefore, the present study aimed to evaluate *in vivo* the hepaprotective activity of aqueous extract and solvent soluble fractions.

2. MATERILELS AND METHODS

2.2. Plant material

Fresh leaves of *Morinda morindoides* were collected in Kimwenza-Kinshasa in the Democratic Republic of Congo. The plant was identified for the first time in October 1990 in the Institut National d'Etudes et de Recherches en Agronomie (INERA), Faculty of Sciences and Technologies, Department of Biology, University of Kinshasa where a voucher specimen was deposited in the herbarium of this institute.

For the present study, a new batch of the plant material (1 kg) was collected at the same place and dried at room temperature for one week. The resulting dried material was reduced to powder using a mortar and pestle and the resulting powder was kept in a brown bottle hermetically closed before use to avoid any contamination.

Figure 1: *Morinda morindoides* leaves, fruits and stem.

2.3. Preparation of extracts and fractionation of aqueous extract

50 g of fresh *M. morindoides* leaves were mixed with 200 mL distilled water and heated on a hot plate at 100°C for 15 minutes. After cooling and filtration on sterile cotton and paper filter Whatman N° 1, the resulting filtrate was evaporated in a rotary evaporator giving a dried extract (28.13 g, denoted as Mms-1).

Next, 15 g of this extract Mms-1 was dissolved in 200 mL distilled water and treated as described above. The resulting filtrate was exhaustively and successively extracted with solvents of different polarities, including chloroform, ethyl acetate and *n*-butanol. All fractions including the residual aqueous fraction were treated as described above resulting in corresponding dried extracts denoted as Mms-1.1 (2.15 g), Mms-1.2 (2.65 g), Mms-1.3 (2.52 g) and Mms-1.4 (4.25 g) for chloroform, ethyl acetate, *n*-butanol and residual aqueous solvent fractions respectively.

2.3. Extraction of polysaccharides

300 g of powdered *M. morindoides* leaves were macerated with 500 mL distilled water for 24 h. After filtration on filter Whatman N°1, the filtrate was concentrated *in vacuo* to 30 mL. To this concentrated aqueous solution, 100 mL of ethanol 90% was added and stored at 4°C for 48 h. Upon storage, a white precipitate was obtained, which was washed with ethanol and dried in an oven at 50°C for one day giving dried extract (CPM: 26.34 g). Upon addition of phenol/H_2SO_4 conc., it showed a positive reaction for the presence of polysaccharides (Trease and Evans, 1989, Harborne 1998, Soniamol et al., (2011).

2.4. Experimental animals

Twenty one healthy adult Swiss albino mice weighing 25-40 g body weight and 5-8 weeks of age (weighing 20-30 g), were purchased from Institut National de Recherches Biomedicales (INRB) in Kinshasa. All animals were fed with commercially available nutrient pellets and had access to water *ad libitum*. The mice were acclimatized for one week prior to the experiment. All mice used in the present study were handled in accordance with the internationally accepted standard guidelines for the use of laboratory animals.

2.5. Evaluation of hepatoprotective activity of extracts, solvent fractions and polysaccharides

2.5.1. Induction of hepatotoxicity and treatment

The induction of hepatoxicity was provoked by using 20% CCl_4)(Merck, Germany) in olive oil and administered by intraperitoneal injection at a dose of 1.5 mL/kg bodyweight (bw) to 21 Swiss albino mice from Institut National de Recherches Biomedicales (INERB) in Knshasa, DRCongo.

2.5.2. Treatment of mice

The 21 mice were grouped as followed:

- Group I was orally administered 5 mL distilled water as normal negative control,
- Group II was injected i.p. 1.5 ml$LCCl_4$/kg bw as toxic negative control to all mice,

- Group III was orally administered Silymarin (100 mg/kg bw) as positive control,
- Group IV and IXa and b were orally administered 200 (a) and 400 (b) mg/kg bw of aqueous extract Mms-1 and methanolic extract Mms-2 respectively,
- Groups VI to Xa and b were administered by the same way the same oral doses of solvent fractions chloroform, ethyl acetate, *n*-butanol and residual aqueous Mms-1.1 to 1.4 respectively,
- Group XI was orally administered the same doses of crude polysaccharides.

CCl_4 20% was injected intraperitoneally to all mice at a dose of 1.5 mL/kg bw after 24 h for intoxication. All mice were provided drinking water *ad libitum* and kept ²on standard pellet diet. (Adeneye et al., 2009, Meharie et al., 2020, Ayenew and Wasihum, 2023, Vouffo et al., 2023, Thodi et al., 2025).

2.6. Determination of time-course effects of extracts and soluble fractions

Time-course in hepatoprotective activity refers to the study of how the protective effect of a substance on the liver changes over a specific duration, analyzing the relationship between treatment time, liver function recovery, and the mitigation of injury. It helps to determine not just if a substance works, but when it works best (e.g., in acute vs. chronic phases) and how long it takes to achieve maximum efficacy.

To determine the time needed for treated samples to offer the maximum hepatoprotection, all intoxicated Swiss albino mice were randomly divided into 4 groups consisting of 3 mice each:

- Group I served as negative control administered 5 ml distilled water,
- Group II and IIIa and b received aqueous Mms-1 and methanol Mm-2 extracts at oral doses of 200 (a) and 400 (b)mg/kg bw respectively,
- Group IV a and b to VIIa and b received the same oral doses by the same way of Mm-1.1 to 1.4 chloroform, ethylacetate, *n*-butanol and residual aqueous phase soluble factions respectively,
- GroupVIII was administered the same oral doses to crude polysaccharides.

All administrations were performed at 1 to 3 h pre and post-CCl_4 induction respectively. (Adeneye et al., 2009).

2.8. Acute and subacute toxicity

Acute and subacute toxicity were assessed using the methods described by OECD (Organization for Economic Co-operation and Development), guidelines 425 and 407, respectively. The mice were fasted for three hours before and one hour after receiving a high oral dose of 5000 mg/kg bw once for the acute toxicity study and 500, 2000 and 5000 mg/kg bw daily in the subacute toxicity study. The mice were placed in separate cages an were observed for signs of toxicity for 28 days.

2.9. Histopathological examination

The method used was previously described by Boro et al. (2022) and Chen and Wang (2025). Mice were killed by sticking**,** animal's neck was cut using a very sharp knife. Briefly, a 5 cm portion of heart, pancreas, lunch, liver and kidney were excised and plentifully washed with normal saline (0.9% NaCl). The tissues were fixed in 10% buffered neutral formalin for at least 48 h, after dehydrated in ethanol 90%, cleared in xylene and embedded in paraffin. Next, 5 µm sections were prepared using a microtome. Then, the liver sections were dewaxed in xylene, rehydrated in a series of different grades of ethanol and then washed with distilled water for 5 minutes. The organ sections were stained with basic stain hematoxylin for 40 seconds and counterstained with acidic stain eosin for 20 seconds (H-E) dye. The sections were examined microscopically (100x and 400x) for any histopathological changes, including cell necrosis, fatty changes and vacuolation. After, all organs were plenty washed with distilled water, dried un an hot at 50°C for 3 days and weighted.

3. REULTS AND DISCUSSION

3.1. Effects of Silymarin, extracts, solvent fractions and crude polysaccharides on biochemicals enzymes

As expected, the intraperitoneal injection of CCl_4 dramatically increased hepatic enzymes like alanine transaminase (ALT and aspartate transaminase (AST), alkaline phosphate (ALP°) gluthathione (GSH), superoxide dismutase (SOD) catalase (CAT), and decreased albumin (ALB) and total proteins (TP) (Table 1a).

However, the oral administration of extracts, solvent fractions and crude polysaccharides from *M. morindoides* leaves to CCl_4-treated mice caused a significant reduction of the levels of these enzymes and products in a dose-dependent manner (Table 1a). Administered at a high oral dose of 400 mg/kg bw, the aqueous extract Mm-1 and methanol extract Mm-2 decreased the levels of these enzymes to 30.55±0.03, 34.32±0.05, 123.13±0.10, 67.56±0.05, 14.67±0.08, to 28.73±0.04, 32.52±0.05, 110.56±0.06, 4.12±0.07, 60.67±0.10 and 10.51±0.08 U/L for ALT, AST, ALP, GSH, SOD and CAT and increased the levels of albumin (ALB) and total proteins (TP) of 5.22±0.13 and 9.02±0.12 mg/dL, respectively. Similarly, oral administration of Mm-1.1 to 1.4 solvent fractions at high dose of 400 mg/kg bw caused also significantly decrease levels of these enzymes to values ranging from 57.46±0.04 to 140.05±0.22 IU/L for all.

Table 1a: Effects of 200 and 400 mg/kg bw oral doses of aqueous extract Mm-1 and its solvent fractions Mm-1.1 to 1.4, methanol 80% Mm-2 extract and crude polysaccharides CPMms on biochemical parameters in CCl_4-induced hepatotoxicity (a).

Extracts and fractions	Treatment	ALT	AST	ALB	TP
NNc	5 Ml DW	22.45±0.05	26.32±0.07	4.12±0.10	8.06±0.12
TNCcCCl$_4$	DW	122.55±0.03	163.15±0.03	2.72±0.03	5.22±0.06
Mm-1 + CCl$_4$	200	71.24±0.06	85.25±0.04	4.83±0.04	7.55±0.04
	400	30.55±0.03	34.32±0.05	5.22±0.13	9.02±0.12
Mms-1.1+ CCl$_4$	200	80.45±0.12	81.25±0.04	5.08±1.20	5.87±0.04
	400	76.62±0.11	78.64±0.09	4.87±0.05	6.35±0.10
Mms-1.2+ CCl$_4$	200	65.11±0.06	68.16±0.10	5.43±0.13	6.22±0.07
	400	57.46±0.05	62.24±0.06	5.75±0.04	7.05±0.11
Mms-1.3+ CCl$_4$	200	85.34±0.05	91.55±0.04	477±0.06	5.92±0.05
	400	80.60±0.06	88.46±0.07	4.66±0.11	6.62±0.04
Mms-1.4+ CCl$_4$	200	72.58±0.08	76.56±0.08	3.53±0.00	5.76±0.12
	400	65.45±0.05	70.07±0.03	5.65±1.21	7.23±0.08
Mm-2+ CCl$_4$	200	68.65±0.06	75.35±0.06	5.70±0.06	8.66±0.07
	400	28.73±0.04	32.52±0.05	6.42±0.06	11.55±0.05
CPMms	200	67.65±0.06	73.35±0.06	3.68±0.06	9.16±0.07
	400	26.75±0.05	30.62±0.05	4.42±0.07	13.35±0.06
Silymarin +CCl4	100	24.46±0.03	30.35±0.06	5.83±0.02	7.85±0.04

DW: distilled water, Mm + CCL$_4$: intoxicated mice treated with *Morinda morindoides* samples, Mm-1: aqueous extract, Mms-1.1 to 1.4: solvent chloroform, ethyl acetate, *n*-butanol and residual aqueous respectively, Mm-2: methanol 80% extract, CPMms: crude polysaccharides.

Selected biochemical enzymes and increased the levels of ALB and TP to values of 5.22±0.13 and 9.02±0.12 mg/dL for ALB and TP respectively.

In Table 1b, it was perceived that the oral administration of Silymarin (25 mg/kg bw) as reference hepatoprotective agent resulted in a significant decrease all selected biochemical parameters alkaline phosphate (ALP), glutathione (GSH) superoxide oxide (SOD) and catalase (CAT) to100.05±0.14, 4.03±0.04, 55.87±0.12, 8.12±0.08 IU/L respectively and decrease of creatinine to 1.34±0.05 mg/mL (Table 1b) compared to toxic CCl_4 negative control, induced significant increase of these parameters (Table 1b). Aqueous and methanol 80% extracts Mms-1 and Mms-2 extracts administered to high oral dose of 400 mg/kg bw reduced also significantly the levels of the biochemical enzymes to 112.45±, 4.36±0.05, 62.84±0.1, 11.64±0.07, and 110.56±0.21, 4.12±0.07, 60.67±0.10, 10.51 IU/L respectively and decrease remarkably creatine to values of 2.23±0.06 and 2.07±0.07 mg/mL respectively. Mms-1.1 to 1.4 solvent fractions yielded also the levels of these biochemical enzymes from 144.12±0.14 to 162.78±0.14, 5.54±0.08 to 8.23±0.12, 67.85±0.08 to 79.08±0.11, 15.31±0.12 to 19.0.08 IU/L for ALP, GSH, SOD and CAT and decline significatively the levels of creatine to value between 2.54±0.04 and 3.12±0.05 mg/mL. Crude polysaccharides also reduced the levels of these enzymes to values of 98.76±0.22, 4.67±0L08, 62.04±0.12 and 11.08±0.10 for the same enzymes cited above in the same order and decreased creatinine level to 2.26±0.07 mg/mL confronted to toxic CCl_4 producing high levels of these biochemical enzyme and creatinine (Table 1b). Silymarin as reference product, significantly reduced the levels of these selected biochemical enzymes and decreased the level of creatinine (Tables 1b). These effects created by Silymarin, extracts, solvent fractions and crude polysaccharides on biochemical enzymes and some product levels demonstrated that these samples exerted good and interesting hepatoprotective effects.

Table 1b. Effects of Silymarin, aqueous extract Mm-1 and its solvent fractions Mm-1.1 to 1.4, methanol 80% Mm-2 extract and crude polysaccharides CPMms on biochemical parameters in CCl_4-induced hepatotoxicity

Extracts and fractions	Treatment (mg/kg bw)	ALP	GSH	SOD	CAT	Creatinine
NNc	5 ml DW	100.00±0.06	4.01±0.03	57.35±0.11	8.05±0.11	1.53±0.08
TNc CCl$_4$	1.5 mL	327.34±0.12	15.72±0.08	80.13±0.13	29.12±0.7	4.23±0.11
Mm-1+CCl$_4$	100	123±10	6.56±0.05	67.56±0.12	14.67±0.8	2.76±0.04
	200	112.45±0.06	4.36±0.05	62.84±0.10	11.64±0.07	2.23±0.06
Mms-1.1+ CCl$_4$	100	163.45±0.12	9.45±0.08	78.76±0.22	19.56±0.08	3.02±0/05
		160.32±0.10	7.67±0.08	74.78±0.18	17.43±0.10	2.45±0.03
Mms-1.2+ CCl$_4$	100	153.14±0.12	7.34±0.06	73.45±0.12	17.86±0.08	3.06±0.06
	200	144.12±0.14	5.54±0.08	67.85±0.08	15.31±0.12	2.54±0.04
Mms-1.3+ CCl$_4$		165.05±0.22	11.56±0.09	82.78±0.13	21.34±0.05	3.87±0.08
		162.78±0.14	8.23±0.12	79.08±0.11	19.56±0.08	3.04±0.05

Mms-1.4+ CCl_4	100	155.10±0.4	6.56±0.12	77.78±0.13	19.68±0.13	3.22±0.05
	200	150.54±0.06	6.04±0.08	71.67±0.11	16.78±0.08	3.12±0.05
Mm-2+ CCl_4	100	120.04±0.12	5.76±0.05	64.03±0.07	12.67±0.10	2.45±0.08
	200	110.56±0.21	4.12±0.07	60.67±0.10	10.51±0.08	2.07±0.07
CPMms		98.76±0.22	4.67±0.08	62.04±0.12	11.08±0.	2.26±0.07
		95.86±0.15	4.06±0.05	58.56±0.10	9.07±0.11	1.91±0.05
Silymarin+CCl4	100	100.05±0.14	4.03±0.04	55.87±0.12	8.12±0.08	1.34±0.05

See Table 1a, NNc: normal negative control, $TNcCCl_4$: negative toxic control. ALP: alkaline phosphate, GSH: glutathione, CAT: catalase, SOD: superoxide dismutase.
Moreover, the levels of all selected biochemical enzymes, and some products can be used as indicators of the liver status. Transaminases are cytosolic enzymes that contribute to the metabolic activity of the liver. The elevated plasma levels of these enzymes indicated abnormal hepatocyte functioning.

Our results displayed a significant increased level of all selected biochemical enzymes and some products in the CCl_4-treated group when compared to normal negative control group, in line with previous studies (Karwani et al., 2015, Ragab et al., 2019, Vouffo et al., 2023). Subsequently, CCl_4 in the Mms treated mice induced significant decreased of levels of all selected biochemical enzymes and some products such as triglycerides urea and uric acid, etc., and increased levels of some products like albumin and total proteins compared to toxic negative CCl_4-treated control (Table 2).

The oral administration of Silymarin led to a significant reduction of levels of these enzymes compared to the negative control CCl_4-treated mice ($TNcCCl_4$ mice) (Table 1a an b)). It is hypothesized that Silymarin protected the plasma membranes from CCl_4-induced damages because of its antioxidant power and that it modified the membrane permeability of the hepatocyte plasma membrane and increased its stability against damages by xenobiotics. Finally, it increased ribosomal RNA by stimulating DNA polymerase 1 and affecting DNA transcription (Kondeva-Burdina et al., 2023). In the context of hepatoprotective activity (the ability of a substance to prevent or repair liver damage).

On the one hand, the aqueous extract Mms-1 and methanol Mm-2 extracts, as well as solvent fractions and crude polysaccharides of *M. morindoides* administered at 400 mg/kg bw to CCl_4-treated mice significantly reduced these biochemical liver enzyme levels and some products and other were increased compared to the negative toxic control CCl_4 ($TNcCCl_4$ mice) (Table 2).

The antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) aid in the reduction of free radicals and thus protect the hepatic cells against oxidant-mediated damage as previously reported by Boro et al., 2022). Our results were in agreement with Kawani, 2015, Boro et al., 2022, Saidurrahman et al. 2022.

3.2. Influences of extracts, solvent fractions and crude polysaccharides on creatinine, uric acid and urea levels

Normal negative control (NNc) mice showed levels of creatinine, urea and uric acid of 6.03±012 mg/mL, 0.65±0.04 g/L and 12.45±1.34 mg/L respectively, which is lower compared to toxic CCl_4-treated mice showing high levels of 6.12±15, 0.71±0.06 and 14.04±1.12 mg/L for the same biochemical parameters above respectively.

The oral administration of Silymarin significantly decreased the levels of creatinine, urea and uric acid at levels of 5.53±0.08 mg/mL, 0.52±0.05 g/L and 11.23±0.07 mg/L respectively, while CCl_4.treated mice showed high levels of 6.12±0.15 mg/mL, 0.71±0.06 g/L and 10.04±1.12 mg/L respectively.

In the same line, the oral administration of aqueous Mms-1 and methanol Mm-2 extracts at a dose of 200 mg/kg bw, caused also remarkable reduction of the levels of these biochemical parameters to respective values of 5.25±0.05 and 5.17±0.08 g/L for creatinine, 5.17±0.08 and 5.13±0.06 g/mL for urea and, 6.43±0.15 and 6.18±0.07 mg/L for uric acid. Solvent fractions Mm-1.1 to 1.4 also significantly reduced the levels of these biochemical parameters to values.

Table 6: Effects of extracts, solvent fractions and crude polysaccharides on creatinine, uric acid and urea levels.

Groups	Treatment	Creatinine (mg/mL)	Urea (g/L)	Uric acid (mg/L)
NNc	5 mL DW	6.03±0.12	0.65±0.04	12.45±1.34
$TNcCCl_4$	1,5 mL	6.12±0.15	0.71±0.06	14.04±1.12
Silymarin	20	5.53±0.08	0.52±0.05	5.23±0.07
Mms-1	100	5.73±0.03	5.47±0.08	7.14±0.12
	200	5.25±0.05	5.17±0.08	6.43±0.15
Mms-1.1	100	5.83±0.15	6.01±0.23	8.53±0.12
	200	5.70±0.03	5.87±0.11	7.65±0.08
Mms-1.2	100	5.85±0.15	5.51±0.22	8.03±0.07
	200	5.47±0.21	5.27±0.15	6.65±0.13

Mms-1.3	100	5.92±0.013	6.11±0.15	8.62±0.06
	200	5.54±0.14	5.82±0.08	6.71±0.11
Mms-1.4	100	5.91±0.12	5.47±0.07	8.12±0.04
	200	5.64±0.11	5.30±0.07	7.73±0.08
Mms-2	100	5.54±0.08	5.36±0.10	7.11±0.14
	200	5.12±0.12	5.13±0.06	6.18±0.07
CPMm	100	5.45±0.04	5.24±0.13	7.05±0.15
	200	5.22±0.07	5.11±0.11	5.75±0.05

See Table 1a, ranging from 5.47±0.21 to 5.70±0.03 mg/mL for creatinine, from 5.13±0.06 to 5.87±0.11 g/L for urea and from 6.65±0.13 to 7.65±0.08 mg/L for uric acid at the same high oral dose.

3.3. Time-courses induced by Silyamrin, extracts, solvent fractions and crude polysaccharides

Results in Table 3 showed the time-course of samples from *M. morindoides* on ALT, AST and ALP recovered in 3h. Results revealed that negative toxic control CCl_4 produced high time-course in intoxicated mice (Table 3). But, aqueous Mms-1 and methanol Mm-2 extracts, and solvent fractions Mm-1.1 to 1.4 at an oral dose of 200 mg/kg bw, induced remarkable prevention of the increase of these parameter levels since these levels were significantly reduced compared to those produced by the negative control toxic CCl_4-treated mice after 3h (Table 3).

Crude polysaccharides at a high oral dose of 400 mg/kg bw reduced also considerably time-course confronted the negative control (Table 3). Silymarin, also decreased significantly these time-course to low values compared to those shown by extracts and solvent fractions from *M. morindoides*. This compound as reference product showed its strong activity after the same The effect of CCl_4 on the liver was similar to that induced by viral hepatitis and paracetamol (Ponmari et al., 2014, Ouassou et al., 2021).

The increased levels of ALT, AST an ALP had been related to the damaged structural integrity of the liver because they were cytoplasmic in origin and were released into the blood after hepatic damages (Ouassou et al., 2021). Our study showed that the activities of all liver enzymes were increased in CCl_4-treated mice. This increase in hepatic markers had been attributed to cell damage or cell membranes losing their integrity (Kharchoufa et al., 2020, Ouassou et al., 2021).

Table 3: Time-course effect induced by of extracts, solvent fractions and Silymarin.

Extracts and fractions	Time-course (h)	ALT	AST	ALP
NNc	3	11.23±0.08	16.04±0.11	24.03±0.06
TNcCCl_4	3	120.85±0.04	165.35±0.03	145.32±0.06
Mms-1	1	32.52±0.06	35.65±0.01	45.21±0.04
	3	42.56±0.08	55.63±0.10	41.03±0.03
Mms-1.1	1	56.05±0.04	60.21±0.07	57.95±0.05
	3	48.69±0.10	56.13±0.00	55.87±0.02
Mms -1.2	1	45.65±0.06	49.03±0.05	50.02±0.05
	3	38.65±1.23	42.85±0.12	47.85±0.01
Mms -1.3	1	77.35±1.04	80.26±1.15	65.66±0.03
	3	68.56±1.02	75.41±0.03	63.71±0.00
Mms -1.4	1	52.62±1.08	65.23±0.11	52.36±0.04
	3	42.65±1.12	55.74±1.30	50.74±0.07
Mms-2	1	37.32±0.05	45.02±0.12	42.65±0.04
	3	28.65±0.11	32.65±0.07	39.87±0.06
CPMms	3	22.45±0.06	30.35±0.04	36.76±0.08
Silymarin	3	12.34±0.04	15.21±0.6	22.05±0.07

See Table 1a, ALT: alanine transaminase, AST: aspartate transaminase, ALP: alkaline phosphatase.

3.4. Influences of extracts, solvent fractions and crude polysaccharides on hematological parameters of CCl_4 + Mms mice

3.3.1. Effects of Silymarin, aqueous extracts, solvent fractions, crude polysaccharides CPMms on triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL) and platelets in intoxicated Swiss albino mice with CCl_4

Results indicated that the intraperitoneal injection of CCl_4 induced significant elevation of the levels of triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL) and platelets compared to normal negative controls (Table 5).

Administration of the aqueous extract Mm-1, 80% methanol extract Mm-2 and soluble fractions Mms 1.1 to 1.4 led to significant decrease of TG, TC, and LDL and increased platelets , total cholesterol and HDL compared

to the negative CCl_4-treated control (Table 5). The effects presented by total cholesterol and lipoproteins types suggested that they may contribute to the prevention of the occurrence of degenerative diseases, particularly cardiovascular diseases (Tshodi et al., 2020, Cimanga et al., 2025).

CCl_4 injection caused significant decrease of platelets, which may hamper blood coagulation. *M. morindoides* leaves samples and Silymarin elevated the levels of platelets in treated mice compared to toxic negative CCl_4-treated group, which produced less platelets (Table 5). Similar effects were also previously reported in other studies (Vouffo et al., 2023).

Table 4: Effects of aqueous extract Mms-1, 80% methanol extract Mms-2, solvent fractions Mms-1.1 to 1.4 and crude polysaccharides CPMms on triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL) and platelets in intoxicated Swiss albino mice with CCl_4

Extracts and fractions	TG	TC	LDL	HDL	Platelets
Normal control	45.23±0.05	38.12±0.03	13.87±0.07	15.45±0.12	26.06±0.14
NT control CCl_4	103.26±0.11	120.75±0.06	20.55±0.04	22.67±0.04	38.34±0.08
Mms-1	86.61±0.04	71.54±1.20	15.55±0.08	25.55±0.12	43.44±0.05
Mms-1.1	78.56±0.10	68.60±0.08	17.65±1.10	25.52±0.05	50.24±0.07
Mms-1.2	84.41±0.07	70.54±0.06	13.46±1.13	24.75±0.05	46.43±0.07
Mms-1.3	76.57±0.10	67.43±0.08	19.66±0.06	24.35±0.07	53.06±0.06
Mms-1.4	82.57±0.05	75.02±1.13	14.34±1.05	24.34±0.08	45.78±0.03
Mms-2	82.41±0.07	73.24±0.08	13.68±0.05	28.24±0.04	40.67±0..04
CPMms	90.06±0.04	78.45±0.06	22.54±0.03	30.15±0.06	52.06±0.07
Silymarin	72.45±0.06	82.32±0.04	11.45±0.06	20.34±0.06	41.23±0.12

See Table 1a, Normal control, negative toxic CCl_4, TG: triglycerides, TC: total cholesterol, LDL: low density lipoprotein, HDL: high density lipoprotein.

Overall, the protective effects of *M. morindoides* leaves on the measured selected biochemical enzymes and some products were significant ($p < 0.01$) since their levels were put back in ranges necessary for the manifestation of hepaprotective effects. In general, from these results, test samples from this medicinal plant part possessed protective effects on the liver and methanol 80% extract Mm-2 and ethyl acetate Mm-1.2 solvent fraction were the better producers of this hepatoprotective activity.

3.3.2. Effects on fasting blood glucose (FBG)

The injection of CCl_4 in mice with streptozocine, resulted in high levels of fasting blood glucose (FBG) of 68.25±0.04mg/dL on Day-0 which continued to increase gradually until day 7 to 81±0.6 mg/dL, which is due to the destruction of pancreatic beta-cells.

By the administration of aqueous extract Mms-1 and methanol extract Mms-2 to CCl_4-treated groups, both extracts caused significant decrease of FBG to values of 66.05±.08 and 64.58±0.06 on day-3 and progressively further decreased to levels of 44.03± 0.03 and 42.03±0.06 mg/dL on day 7, while, negative toxic CCl_4-treated mice showed a high level of 81.06±0.05 mg/dL.

Treatment with fractions resulted in FBG levels ranging from 66.02±1.1 to 65.75±0.14 mg/dL on day 0, reducing FBG level of CCl_4 + Mms mice to values from 63.56±1.03 to 65.25±1.20 mg/dL on day-3, and continuing progressively to reach lower values between 47.65±0.08 and 49.65±0.04 mg/dL on day 7 in CCl_4-treated +Mms mice, while crude polysaccharide administration led to aFBG of 60.45±0.03 on day 0, caused its reduction to 58.82±0.07 mg/dL on day 3, to attain a low value of 38.55±0.04 mg/dL on day 7, comparedto the negative CCl_4-treated mice with an FBG level of 81.06±0.05 mg/dL.

The effects shown by extracts, solvent fractions and crude polysaccharides may be due to their hypoglycemic properties as also reported for other medicinal plant extracts (Tshodi et al., 2022, Cimanga et al. 2025). Glibenclamide used as reference hypoglycemic drug caused also significant decrease of FBG on intoxicated mice with FBG of 20.45±0.05 mg/dL on Da0 and known progressive slope to attain finally level of 6.45±0.04 mg/Dl on Day-7. For this hypoglycemic test in intoxicated mice, it was observed that methanol Mms-2 extract showed high activity compared to aqueous Mms-1 extract and the ethyl acetate Mms-1.2 exhibited a high activity compared to chloroform Mms-1.1, *n*-butanol Mm-1.3 and residual aqueous solvent fractions related to the nature of active principles that they contain (Tshodi et al, 2020, Tegegne et al., 2023, Cimanga et al, 2025b).

Table 5: Effect of extracts and soluble fractions on the fasting blood glucose (FBG)

CCl_4-pretreatment FBG (mg/dl)				72 h post CCl_4 treatment FBG (mg/dl)
Extracts/Fractions	Treatment	Day-0	Day-3	Day-7
NNc	5Ml DW	21.12±0.07	17.04±0.05	4.87±0.04
TNcCCl_4	5 mL DW	68.25±0.04	75.15±0.02	81.06±0.05
Glibenclamide	5	20.45±0.05	15.05±0.07	6.45±0.04

Mms-1	200	66.35±0.04	70.56±0.05	56.52±0.08
	400	68.52±0.06	64.05±0.08	44.03±0.03
Mms-1.1	200	67.84±0.07	66.25±0.2	60.54±1.12
	400	66.22±0.04	65.25±1.20	55.41±1.02
Mms-1.2	200	65.84±0.08	64.65±0.05	52.36±0.13
	400	66.02±1.11	63.56±1.03	47.65±0.08
Mms-1.3	200	65.78±0.06	64.85±0.03	67.35±0.05
	400	66.34±0.12	65.36±0.08	62.54±1.02
Mms-1.4	200	64.95±1.30	64.85±0.09	55.62±0.03
	400	65.75±0.14	64.15±1.10	49.65±0.04
Mms-2	200	66.23±1.21	69.45±1.14	52.65±0.07
	400	66.05±0.08	64.58±0.06	42.03±0.06
CPm	200	62.56±0.06	60.75±0.04	40.85±0.08
	400	60.45±0.03	58.82±0.07	38.55±0.04

See Table 1a.
Glibenclamide was a medicine bought in Pharmacie La Quinine in Kinshasa, RDCongo.

In general, all effects shown by extracts, solvent fractions and crude polysaccharides as well as Silymarin on hepatic enzymes, biochemical and hematological products as well as their effects on time-course and impact on functional integrity of cell membranes, clearly demonstrated that these samples exerted hepatoprotective effects towards the liver. Our results were in good accordance with other studies who have also reported the hepatoprotective activity of several medicinal plant extracts and solvent fractions (Ubhenin eal, 2016, Meharie et al, 2020, Kopolakkal et al, 2021, Ouassou et al., 2021, Boro et al., 2022, Tegegne et al., 2023, Cimanga et al., 2025).

The major mechanism of action of medicinal plant extracts in the protection of liver cells and to prevent the occurrence of liver injury liver was the eradication of free radicals, reducing oxidative stress and decreasing the proinflammatory cytokine mediators in the body. The most probable mechanism of action of several medicinal plants was through the scavenging effect of harmful free radicals generated (Sumaia et al., 2019, Pandey et al., 2023) or again, the mechanism of action as hepatoprotective agents might be due the antioxidant properties of medicinal plant species which had major role in protecting liver cells from oxidative stress (Sumaia et al., 2019). Based on this observation, it was assumed that extracts and solvent fractions from *M. morindoides* leaves exerted these hepatoprotective effects by their free radical scavenging activity / their antioxidant activity, which was already demonstrated in a previous study on a large set of reactive oxygen species (ROS) (Cos et al., 1998, Cimanga et al., 1999, 2021) as well as in view of its anti-inflammatory and analgesic activities (Cimanga et al., 2025a).

Moreover, previous data showed that alkaloids, coumarins, flavonoids, steroids, terpenoids polysaccharides, saponins and tannins are known as hepatoprotective agents and the presence of these phytochemical groups in *M. morindoides* leaves could contribute to the manifestation of this biological activity (Cimanga at al., 2006a).

Secondary metabolites, such as phenolics (flavonoids, coumarins, lignans), terpenoids, and alkaloids, act as hepatoprotective agents primarily by neutralizing free radicals, reducing lipid peroxidation, and stabilizing cell membranes. They work by inhibiting CYP450 enzymes to reduce toxic metabolite production, suppressing inflammatory pathways (NF-κB, MAPK), improving antioxidant enzymes (SOD, GSH), and inhibiting apoptosis.

Their key mechanisms of action included

- Antioxidant and Free Radical Scavenging: Metabolites (e.g., silymarin, flavonoids) neutralize reactive oxygen species (ROS) produced by toxins like alcohol or carbon tetrachloride (CCl_4).
- Membrane Stabilization: They maintain the structural integrity of hepatocyte membranes, preventing the leakage of liver enzymes (ALT, AST).
- Anti-inflammatory Effects: Compounds (e.g., andrographolide) inhibit the release of pro-inflammatory cytokines and inflammatory pathways such as *NF-$_k$B* and *MAPK*.
- Anti-apoptotic Activities: Triterpenoids and other compounds suppress the expression of caspases, inhibiting cell death.
- Metabolic Regulation (Cytochrome P450 Inhibition): Certain compounds inhibit, reducing the conversion of xenobiotics into toxic metabolites.
- Antifibrotic & Regenerative: They reduce liver fibrosis and promote liver cell regeneration.

MDPI +5Key Secondary Metabolites and Sources

- Flavonoids/Flavonolignans (e.g., Silymarin from *Silybum marianum*): Known for antioxidant, membrane-stabilizing, and anti-inflammatory properties.
- Terpenoids (e.g., Glycyrrhizin from *Glycyrrhiza glabra*): Act as potent anti-inflammatory and antioxidant agents.

- Phenolic Compounds (e.g., Curcumin from *Curcuma longa*): Known to modulate detoxification enzymes.
- Alkaloids: Some exhibit strong anti-inflammatory and hepatoprotective properties.

Their mechanisms of action are mainly to inhibit lipid peroxidation, promote the recovery of the liver cell membrane, eliminate ox...

The hepatoprotective and antioxidant activity of silymarin is caused by its ability to inhibit the free radicals that are produced from the metabolism of toxic substances such as ethanol, acetaminophen, and carbon tetrachloride. The generation of free radicals is known to damage cellular membranes and cause lipoperoxidation. Silymarin enhances hepatic glutathione and may contribute to the antioxidant defense of the liver. It has also been shown that silymarin increases protein synthesis in hepatocytes by stimulating RNA polymerase I activity. A previous study on humans reported that silymarin treatment caused a slight increase in the survival of patients with cirrhotic alcoholism compared with untreated controls (Anonymous, 2025b).

3.1.1. Acute and subacute toxicities

The body weight of CCl_4-treated mice was compared to the normal negative control group. Results revealed that CCl_4-treated mice showed a considerably reduction in body weight compared to normal negative control group. Inversely, CCl_4-treated mice receiving *M. morindoides* leaf extract at an oral dose of 5000 mg/kg bw once in acute toxicity gained body.

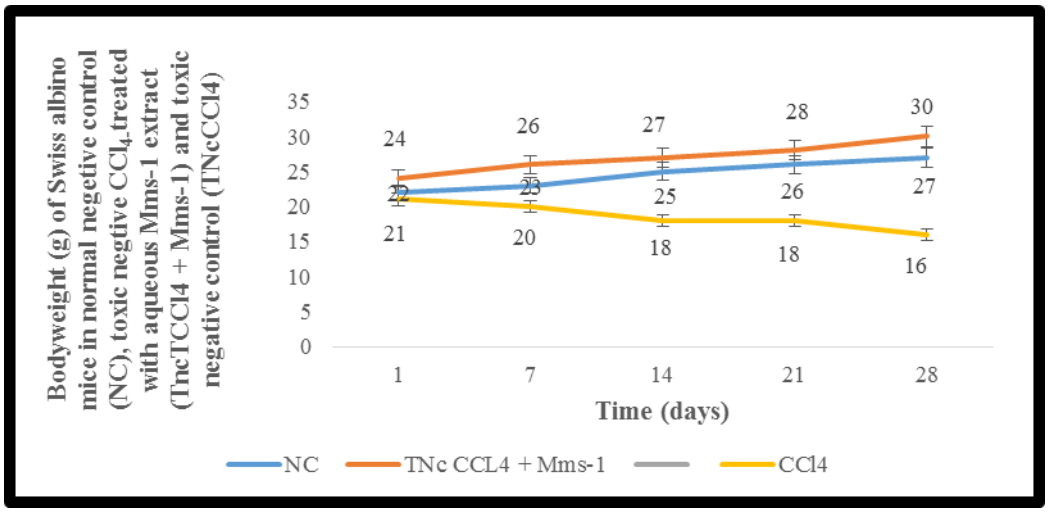

Figure 2: Body weight (g) of normal negative control mice, negative CCl_4-treated mice and CCl_4-treated mice administered 400 mg aqueous extract of *M. morindoides* leaves (CCl_4 + Mms mice).

Weight compared to CCl_4-treated and normal negative control mice as illustrated in Figure 2. It was observed that the oral administration of aqueous extract of *M. morindoides* leaves prevents the loss of body weight of CCl_4-treated mice, since these later gained bodyweight compared to normal negative and toxic negative control CCl4 treated groups with aqueous Mms-1 extract due to hepatoprotective effects off aqueous Mms-1 extract from *M. morindoides* leaves (Fig. 2). It significantly improved the growth of CCl_4-treated mice compared to toxic negative control CCl_4 mice group. This increase in body weight by CCl_4-treated mice was previously also reported by Meharie et al. (2020) for the 80% methanol extract of *Clutia abyssinica* leaf and our finding was in good agreement with these previously reported results.

3.1.2. Effects of *M. morindoides* on vital organs

Vital organs like heart, liven kidney, lunch and pancreas of intoxicated mice were removed, washed plentifully with distilled water and dried in a oven at 50°C until constant weight. It was found that CCl_4-treated mice induced a high weight of all selected vital organ compared to normal negative control (NC) (Fig.3). The administration of aqueous extract of *M. morindoides* leaves in CCl_4-treated mice resulted in a significant decrease ($p < 0.05$) of weights of all vital organs compared to CCl_4-treated group. The same effect was also observed with the administration of Silymarin as reference hepatoprotective agent (Fig. 3).

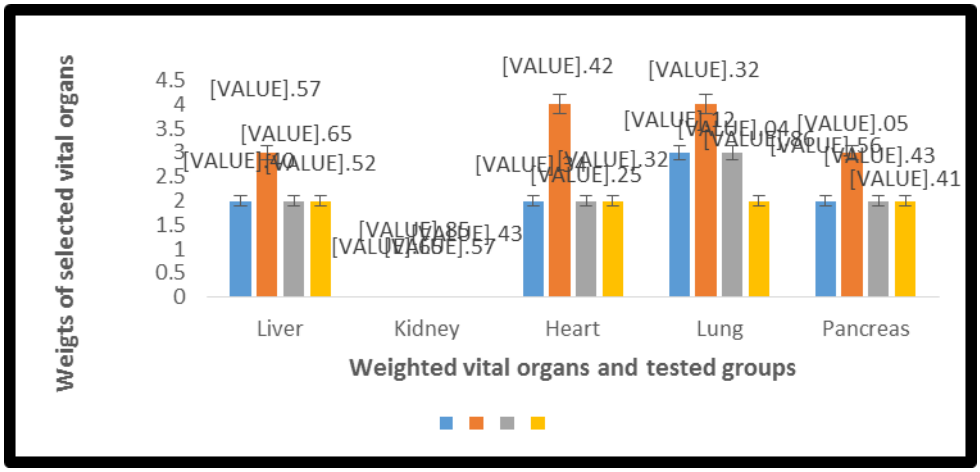

Figure 3: Weights of vital organs.

The effect of the aqueous extract from *M. morinodoides* leaves of the liver architecture showed no significant difference compared to normal negative control (NNc group) suggesting a hepatoprotective effect generate by this administered extract.

2. Histopatological examination

In the histopathological examination of the liver tissue, normal control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein. There were no signs of inflammation and fibrosis. Blood vessels were normal with no signs of hemorrhage. Whereas CCl_4-treated liver tissues showed signs of toxicity such as necrotic cells, inflammation, hemorrhage, and even fibrosis or fatty changes. Blood vessels appeared normal in CCl_4 group.

In a group treated with silymarin, these signs of toxicity such as necrosis, inflammation, hemorrhage, and fibrosis were not demonstrated. In the group of aqueous extract of *Morinda morindoides* leaves at the high oral dose of 400 mg/kg bw, all these signs of toxicity were absent, the tissue showed normal architecture compared to normal negative control.

Futhermore, the histopathological examination of the liver and kidney provided evidence of *M. morindoides* acting against acute CCl_4-induced liver injuries. This histological investigation showed that CCl_4 induced liver damages with the occurrence of necrosis, inflammation, cytoplasmatic vacuolation, hepatocellular degeneration and loss of cellular boundaries which were in agreement with the previous studies for liver injuries (Vuda et al., 2012, Lopez et al., 2017).

Fortunately, the treatment with aqueous Mms-1 extract of *M. morinodoides* leaves at oral doses of 200 and 400 mg/kg bw reduced the severity of all abnormal manifestations occurring during oxidative damages in a dose-dependent manner. This was marked by a decrease of necrosis, inflammation and other unwanted evenments occurring at high treated dose of *M. morindoides* leaves aqueous extract. Hence, it was shown that the aqueous Mms-1 extract from *Morinda morindoides* leaves showed hepatoprotective effects.

4. CONCLUSION

The current study reported for the first time the *in vivo* hepatoprotective effects of aqueous and methanol 80% extracts, chloroform, ethyl acetate, *n*-butanol and residual aqueous soluble fractions, as well as crude polysaccharides from *M. morindoides* leaves. All test samples exhibited promising hepatoprotective effects. In addition, it was reported in the literature that decreasing the metabolic activation of CCl_4, the antioxidant activity, the prevention of generation of reactive oxygen species and free radical scavenging activity or a combination of these, could be the underlying mechanisms. In earlier studies, the hepatoprotective actions of medicinal plants were reported to be mediated by the presence of secondary metabolites like alkaloids, flavonoids, saponins, tannins, anthraquinones, polysaccharides, etc. or by their combination via antioxidant and free radical scavenging activity. The presence of these classes of constituents in *M. morindoides* leaves (Cimanga et al., 2006a), might be related to the hepatoprotective effects of this plant species, as well as to previously reported antioxidant and free radical scavenging activities of *M.* (Cimanga et al., 1999, 2021b). The present study highlights the potential for the use of *M. morindoide*s leaves as an alternative to treat liver disorders.

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