

Investigation of the Effects of *Monodora myristica* Seeds (Annonaceae) against Liver Dysfunctions and Oxidative Stress on Paracetamol-Intoxicated Wistar Rats

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Abstract

The seeds and bark of *Monodora myristica* are used in traditional Cameroonian medicine to treat various ailments, such as fevers and kidney and liver infections. This study aimed to investigate the effects of aqueous seeds extract of *Monodora myristica* against liver dysfunctions and oxidative stress on paracetamol-intoxicated rats. Thirty rats including males and females were divided into 6 groups of 5 each: Group 1 (normal control) received distilled water (5 mL/kg); Group 2 (negative control) received paracetamol (2 g/kg) and distilled water (5 mL/kg); Group 3 (positive control) received paracetamol (2 g/kg) and silymarin (25 mg/kg); Groups 4, 5 and 6 received paracetamol (2 g/kg) followed by the aqueous seeds extract at doses of 100, 200 and 300 mg/kg, respectively. Our results demonstrated that paracetamol caused significant adverse effects on body weight, biochemical parameters, the oxidant-antioxidant balance, and liver histopathology in negative control rats compared to normal control. Treatment with the aqueous extract at 300 mg/kg significantly increased body weight (154 vs 141 g) compared with the negative control ($p < 0.05$). The extract 300 mg/kg markedly reduced ALT (145.8 vs 114.4 Units/L, $p < 0.05$), AST (145.8 vs 122.0 Units/L, $p < 0.01$), ALP (40.00 vs 34.20 Units/L, $p < 0.05$), and T. Bil (6.60 vs 2.20 Units/L, $p < 0.05$) compared to negative control. Administration of extract (300 mg/kg) significantly decreased, Total cholesterol (58.80 vs 45.60 mg/dL, $p < 0.05$), TG (74.40 vs 60.40 mg/dL, $p < 0.01$), and increased HDL cholesterol (19.00 vs 26.40 mg/dL) compared to the negative control. The extract 200 and 300 mg/kg increased protein activity (1.01 and 1.14 vs 0.86 mg/g respectively) compared to the negative control ($p < 0.01$). The extract 300 mg/kg significantly increased CAT (12.00 vs 16.74

IU/mg of proteins, $p < 0.05$) and SOD (129 vs 247 IU/mg of proteins) compared to the negative control. These results demonstrated that the aqueous extract of *M. myristica* seeds has therapeutic and antioxidant effects.

Keywords

Liver, Paracetamol, Oxidative Stress, *Monodora myristica*

1. Introduction

Liver dysfunction occurs when the liver is damaged and can no longer normally perform its functions of filtering toxins, processing nutrients, and synthesizing proteins. Approximately 2 million people die each year from liver diseases (1 million from complications of cirrhosis and 1 million from viral hepatitis and hepatocellular carcinoma). Today, the number of patients suffering from liver dysfunction is steadily rising due to excessive alcohol consumption, drug use, and auto-medication [1]. Liver dysfunction can range from mild inflammation to life-threatening liver failure. When the liver does not function normally, it can affect the entire body, leading to jaundice, edema, dark urine, pale stools, and cognitive impairment [2]. Liver dysfunction often progresses through distinct stages: inflammation (hepatitis), fibrosis, cirrhosis, and liver failure [3]. Several factors can lead to liver dysfunction, such as viral infections (hepatitis A, B, and C), alcohol consumption and diet (excessive and prolonged alcohol intake or fat accumulation in the liver, known as hepatic steatosis), autoimmune and genetic diseases (conditions in which the immune system attacks the liver or causes the accumulation of harmful substances), medications and toxins [4]. Overdoses of certain medications (such as acetaminophen) or exposure to toxic chemicals cause liver dysfunction.

Paracetamol (acetaminophen, N-acetyl-p-aminophenol) is the most widely used analgesic and antipyretic medication worldwide, available both with and without a prescription [5]. The main toxic effect of paracetamol is hepatotoxicity. It exerts its toxic effect through the toxic reactive metabolite, N-acetyl-benzoquinone imine (NAPQI) [6]. This metabolite is formed by cytochrome P-450 (CYP) enzymes, primarily CYP2E1 and CYP3A4. At normal therapeutic doses, NAPQI is produced in very small quantities and is rapidly detoxified or neutralized through conjugation with glutathione [7]. In an overdose situation, the amount of glutathione may be insufficient to detoxify the toxic metabolite (NAPQI), leading to its binding to cellular proteins; NAPQI binds primarily to cysteine residues but is also able to damage proteins at methionine, tryptophan, and tyrosine residues [8]. The mitochondria are the primary targets for the formation of NAPQI adducts (the binding of a chemical molecule to cellular DNA). The resulting formation of reactive oxygen species induces oxidative stress, which in turn triggers the activation of c-Jun N-terminal kinase (JNK). Once activated, the JNK enzyme (which

acts as a gene expression modifier, primarily at the transcriptional level) migrates to the mitochondria, causing mitochondrial dysfunction, the cessation of ATP production, and disruption of the mitochondrial membrane [9]. Mitochondrial dysfunction leads to cellular necrosis (premature and unplanned cell death). Severe liver injury inevitably leads to a loss of hepatic synthetic function [10]. Clinical signs of hepatotoxicity are altered, with peak serum transaminase levels appearing two to three days after acetaminophen overdose [9].

Once paracetamol is ingested, an acetylation reaction (a chemical reaction involving the introduction of an acetyl group) occurs, creating a primary amine (4-aminophenol). Although this molecule is involved in the formation of an endogenous cannabinoid (which activates CB1 cannabinoid receptors to relieve pain), it is also implicated in the toxic effects of acetaminophen [11]. Excessive accumulation of acetaminophen and 4-aminophenol often leads to liver failure. Approximately 12% - 13% of overdose cases result in hepatotoxicity, even with treatment, 2% - 5% of these cases progress to liver failure, and 0.2% - 0.5% lead to death [12]. Certain medications are used to treat liver dysfunction: The use of amygdalin, dicoumarol, quercetin, and ancient ephedrine decoction to treat hepatitis by inhibiting viral replication, Liuwei Wuling tablet to alleviate acute liver injury by improving oxidative stress and inflammatory responses in the body. Saikosaponin, dehydroabietic acid, hesperetin, berberine, and *Panax Notoginseng* saponins. for metabolic-associated fatty liver disease. Germacrone, forsythin, geniposide, and protocatechuic acid can inhibit the activation or migration of stellate cells and improve liver fibrosis [13]. Silymarin can alleviate biochemical and transaminase levels [14]. Paracetamol is used as an animal model because its toxic mechanism mimics human liver failure and is accompanied by measurable biochemical changes and visible damaged liver architecture [15]. Therefore, paracetamol serves as a control reference for evaluating whether a medicinal plant such as *Monodora myristica* can prevent liver cell death, reduce oxidative stress, and restore normal liver function.

Monodora myristica has been used in Africa for centuries as a medicinal plant to treat various ailments. The plant's bark and seeds are used to reduce fever and treat liver disorders. The seeds are also used as a culinary spice, prized for their flavour and the aroma they release [16]. The seeds, just like the leaves, are used to treat chest and joint pain while its roots and bark are used to relieve digestive disorders. Additionally, the seeds are used to treat bronchitis and coughs [17]. High-performance liquid chromatography (HPLC) analysis of *M. myristica* revealed prominent peaks corresponding to gallic acid, catechin, and quercetin, indicating the presence of phenolic acids, flavan-3-ols, and flavonoids, respectively [18]. HPLC analysis of *Monodora myristica* extract also showed an abundance of myristyl chloride, linalool, nerolidol, elemicin, myristicin, eugenol, and pinene [18]. The analysis of *Monodora myristica* using gas chromatography-mass spectrometry (GC-MS) aimed at identifying its secondary metabolites and characterizing its oil, revealed the presence of metabolites such as saponins, alkaloids, tan-

nins, and flavonoids. Characterization of the oil indicated the presence of organic compounds and fatty acids, specifically palmitic, eicosanoic, stearic, and oleic acids [19]. Given the diversity of chemical compounds found in *Monodora myristica*, we aimed to evaluate the effects of the aqueous extract of *M. myristica* on animal body weight and on certain liver function parameters specifically ALT, AST, ALP, and total bilirubin. We also examined the lipid profile (including total protein, total cholesterol, and HDL and LDL cholesterol) and oxidative stress markers such as CAT, SOD, and MDA, as well as the liver histology of paracetamol-intoxicated animals.

2. Materials and Methods

2.1. Collection and Preparation of Plant Material

The fresh seeds of *Monodora myristica* were collected in Ndengnieup village, Bangou Subdivision in Upper plateau Division, West region Cameroon (5°14'5" North and 10°25'57" East) on the 10th December 2022. The fruits were identified with the help of local herbalists. The information gathered included vernacular name (Maidjeu eu), parts used (root, stem bark, leaves and seeds), quantity used (one coffee spoon of powder in a cup of water), and the ailment treated (jaundice, nervous disorder, infertility, and liver diseases). The plant samples were finally provided to an acknowledged taxonomist for botanical authentication, and the dry plant samples of the *M. myristica* were deposited and kept at room temperature at the University of Bamenda, Cameroon, for future reference. The plant was identified and compared with the material; letouzey Nana P 379 of the National Herbarium of Cameroon, where a voucher specimen is registered under the number No: 2317 SRF/Cam. The seeds of *M. myristica* were chapped into small pieces and air dried away from solar radiation (temperature 22°C - 25°C) to avoid destruction of plant's substance by sun, for two months until properly dried. The dried seeds of *M. myristica* were crushed into fine homogenous powder using electrical mill followed by sieving through mesh sieve and stored at room temperature waiting extraction.

2.2. Extraction of Aqueous Extract of *M. myristica* Seeds

The dried powder (600 g) was macerated in 1.5 liter of distilled water for 72 hours to extract the active compounds (1:2.5) [20]. The successive extract was filtered using Wattman's No. 1 filter papers. The obtained filtrate was evaporated at 40°C to dryness for 72 hours in a thermostat oven (DHG-9101-15APEC) in biochemistry laboratory, University of Bamenda. A dark brown solid extract was obtained, and the mass 86.17 g recorded, representing an extraction yield of 10.77%. It was stored in tight labelled bottle in a refrigerator at 4°C until further use. Extract was dissolved in distilled water and administered by oral route (drinking) to rats. Based on the information from the local herbalists, the doses of 100, 200 and 300 mg/kg body weight (bw) were selected as follows: Herbalists dissolve approximately 5 g spoon of powdered *Monodora myristica* seeds in 500 mL of drinking

water and consume this daily to treat liver disorder. They consume two glasses for a person weighing approximately 70 kg. Therefore, it was carried out for two hours, stirring every 30 minutes. After filtration and evaporation, 1.14 g of extract was obtained. This mass of extract is assumed to be consumed by an adult of 70 kg body weight. Some authors have explained the formula for determining the therapeutic dose in humans [21]. Researchers have developed a formula for the relationship between therapeutic doses in humans and therapeutic doses in animal species [22]. These formulas are as follows:

$$\text{HTD}(\text{mg/kg}) = \frac{\text{Weight of the extract consumed}}{\text{Body weight}}$$

where: HTD = Human therapeutic dose

$$\text{RED}(\text{mg/kg}) = \text{HTD} \times \frac{\text{Human Km}}{\text{Rat Km}}$$

where: RED = Rat equivalent dose, Km = Species standard factor with Human Km = 37 and Rat Km = 6.

Therefore, $\text{HTD} = 1.14 \text{ g}/70\text{kg} = 16.28 \text{ mg/kg}$ and $\text{RED} = 16.28 \times 37/6 = 100 \text{ mg/kg}$.

After obtaining this RED, we multiplied this dose by 2 to find the dose of 200 mg/kg and by 3 to find the dose 300 mg/kg because toxicity needs enough substances to neutralize adverse effects.

2.3. Experimental Animals

Thirty-five (35) male and female Wistar rats (*Rattus norvegicus*) (3 to 4 weeks old, average weight 110 - 125 g) were obtained from breeder at mile 4 Nkwen Bamenda, North-west region Cameroon. The experimental rats were raised in the animal house and kept under standard laboratory conditions (12 hours light and dark sequence, temperature $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$, and 35% - 60% humidity). The animals were caged in plastic cages of dimension (45.5 cm of diameter and 22.5 cm of high), with an appropriate diet (30% weed brown, 30% soya beans, and 40% maize) and water tap *ad libitum* [23]. They were handled by considering to the ethical guidelines of Cameroon national veterinary laboratory as referenced by the approval and head central No 001/24CCS/MINPIA/RD-NW/DD-MELSSV dated 12/01/2023. All the animals were acclimatized for two weeks, before the beginning of the experiment and to ensure that the new substances were completely administered to the rats and the desired dose was in their bodies, food was withdrawn 12 hours before initiation of experiments [24].

2.4. Experimental Design and Treatment of Experimental Animals

After the acclimatization period of two weeks, 30 healthy rats were selected and divided into 6 different groups, comprising 18 males and 12 females. Regarding cage allocation, each group was housed in one cage containing three males and

another containing two females. The groups were named 1 to 6 and designed as follows: group 1, (normal control) received distilled water (5 mL/kg); group 2, (negative control) received paracetamol (2 g/kg) and distilled water (5 mL/kg); group 3, (positive control) received paracetamol (2 g/kg) and silymarin (25 mg/kg); groups 4, 5 and 6 received paracetamol (2 g/kg) followed by the aqueous seeds extract at doses of 100, 200 and 300 mg/kg, respectively. From groups 2 to 6, paracetamol was administrated (2 g/kg) to induce hepatotoxicity every Tuesday, Thursday, and Saturday at 11 am and treatment every Wednesday, Friday and Sunday at 5 am for 4 weeks. At the end of the experiment on the twenty ninth day at 5 am, all the rats were sacrificed by decapitation. Blood samples were collected, centrifuged at 3000 rpm for 15 minutes to obtained clear serum. Serum was stored at -20°C and used for liver functional parameter analyses. The animals were dissected and liver tissues were removed. One part to assess oxidative stress parameters and another part was trimmed down for histological analysis [24].

2.5. Effect of Extract on Body Weight

The groups were treated as described in the experimental design above. The rats were divided into six groups [25]. The rat was treated in the same experimental design for 4 weeks, consecutively and weighted on the week 1, 2, 3 and 4. All the body weight was recorded.

2.6. Analysis of Liver Enzymes

At the end of the experimental period on the twenty ninth day at 5 a.m. all the rats were sacrificed by decapitation. blood samples were collected from the experimental animals under the appropriate anaesthesia/sampling procedure. centrifuged at 3000 rpm for 15 minutes to obtained clear serum. Serum was stored at -20°C until biochemical analysis.

2.6.1. Alanine Aminotransferase

Serum alanine aminotransferase (ALT) activity was determined using the Randox ALT (GPT) reagent kit (catalogue no. AL3801; Randox Laboratories Ltd.). The Randox AL3801 assay is based on the UV method according to the IFCC principle and is intended for the quantitative determination of ALT in serum or plasma. For the kinetic assay, the serum sample was mixed with the appropriate working reagent according to the manufacturer's instructions and incubated at the temperature of 37°C . The change in absorbance was monitored spectrophotometrically at 340 nm, corresponding to the oxidation of NADH to NAD^{+} . ALT activity was calculated from the rate of change in absorbance per minute and expressed as U/L.

2.6.2. Aspartate Aminotransferase

Serum aspartate aminotransferase (AST) activity was determined using the Randox AST reagent kit (catalogue no. AS3804), based on the modified IFCC UV

method. Randox describes these reagents as quantitative assays for AST in serum or plasma. The serum sample was mixed with the working reagent and incubated under the conditions specified by the manufacturer. The enzymatic reaction was monitored kinetically by measuring the decrease in absorbance at 340 nm due to NADH oxidation. AST activity was calculated from the change in absorbance per minute and expressed as U/L.

2.6.3. Alkaline Phosphatase

Serum alkaline phosphatase (ALP) activity was determined using the Randox ALP reagent kit (catalogue no. AP3802). The assay is based on the AMP method and is intended for quantitative determination of ALP activity in serum or plasma. Serum was mixed with the ALP working reagent and incubated according to the manufacturer's specified assay conditions. The formation of the coloured reaction product was monitored spectrophotometrically, generally at approximately 405 nm for the p-nitrophenol-based ALP reaction. The rate of increase in absorbance was used to calculate ALP activity, which was expressed as U/L.

2.6.4. Total Bilirubin

Serum total bilirubin concentration was determined using the Randox Total Bilirubin reagent kit (catalogue no. BR3859), based on the modified Jendrassik method. The BR3859 kit contains liquid reagent R1 and the R2a/R2b components and is intended for the quantitative determination of total bilirubin in serum or plasma. Briefly, serum was reacted with the Randox bilirubin reagents according to the manufacturer's instructions. Bilirubin reacts with diazotised sulphanilic acid to form a coloured azobilirubin complex. Following the specified incubation period, absorbance was measured spectrophotometrically at the wavelength specified in the kit manufacturer's application sheet 578 nm, and total bilirubin concentration was calculated against the appropriate calibration/standard and temperature 20°C to 25°C. Results were expressed in U/L.

2.7. Analysis of Lipid Profile

2.7.1. Total Cholesterol

Serum total cholesterol concentration was determined enzymatically using the Randox Cholesterol reagent kit (catalogue no. CH3810). The CH3810 assay uses the cholesterol oxidase-peroxidase (CHOD-PAP) enzymatic method and is intended for quantitative determination of cholesterol in serum or plasma. Serum was mixed with the cholesterol working reagent and incubated according to the manufacturer's recommended conditions. The enzymatic reaction produces hydrogen peroxide, which subsequently reacts with chromogenic substrates to form a coloured compound. Absorbance was measured spectrophotometrically at the wavelength specified by the manufacturer's application/kit instructions 546 nm, at temperature of 20°C to 25°C. The cholesterol concentration was calculated from the absorbance of the sample relative to the standard/calibrator. Results were expressed in mg/dL.

2.7.2. Triglyceride

Serum triglyceride concentration was determined using the Randox Triglycerides reagent kit (catalogue no. TR3823). The assay is based on the enzymatic GPO-PAP method. Randox describes TR3823 as a quantitative assay for triglycerides in serum or plasma. Serum was reacted with the triglyceride working reagent according to the manufacturer's instructions. Triglycerides were enzymatically hydrolysed to glycerol, followed by a series of enzymatic reactions producing hydrogen peroxide. The latter reacts with chromogenic compounds to generate a coloured product. Absorbance was measured spectrophotometrically at the wavelength specified by the kit application at 546 nm, temperature 37°C and incubation 10 minutes. The triglyceride concentration was calculated from the sample-to-standard absorbance relationship. Results were expressed in mg/dL.

2.7.3. HDL-Cholesterol

Serum HDL-cholesterol (HDL-C) concentration was determined using the Randox HDL-Cholesterol reagent kit (catalogue no. CH3811). This assay uses a direct clearance method and is designed for quantitative determination of HDL-cholesterol in serum without a preliminary precipitation step. Serum was directly reacted with the Randox HDL-C reagents according to the manufacturer's instructions. Non-HDL lipoproteins were selectively masked/removed by the reagent system, while HDL-associated cholesterol was subjected to enzymatic determination. The resulting colour development was measured spectrophotometrically under the wavelength and reaction conditions specified in the manufacturer's application sheet at 600 nm, temperature 37°C, and incubation time 5 minutes. HDL-C concentration was calculated using the appropriate calibration procedure and expressed in mg/dL.

2.7.4. Calculation of Cholesterol Low-Density Lipoproteins

The concentration of LDL-cholesterol was calculated using Friedewald equation [26] as follows:

$$\text{LDL cholesterol} = \text{Total cholesterol} - \frac{\text{triglyceride}}{5} - \text{HDL cholesterol}$$

2.8. Antioxidant Activity and Total Proteins

Measurement of some biomarker on oxidative stress was as follows: the procedure to estimate malondialdehyde (MDA) activity by the method of [27]. The activity of catalase (CAT) in tissues was evaluated by the method of [28]. The superoxide dismutase (SOD) activity was determined in supernatant of homogenate by the method of [29]. The protein content in homogenate was measured using the method described by [30].

2.9. Histological Studies

To study the liver sections under microscope, 10% formalin was used for the fixation of collected liver sample [24]. Later, samples were passed via many proceedings of fixation, sectioning and stain. In fixation, liver samples of all experimental

groups were kept in 10% neutral formalin. After fixation, tissues were dehydrated in different percentages of alcohol (75%, 95% and 100% absolute), embedded in paraffin block and serially sectioned (5 μ m size) using a microtome. Liver sections were stained with mayer hematoxylin and eosin. paracetamol induced liver damage was observed using microscope (Zeiss, Hallherbermoos, Germany).

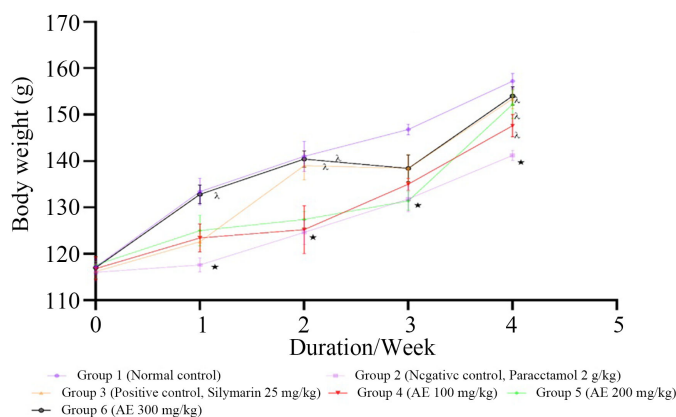
2.10. Statistical Analysis

All data were presented as mean $\pm$ S.E.M of five rats ($n = 5$). Difference between means was assessed by one-way and two-way Analysis of Variance (ANOVA), followed by the Bonferroni post-test using Graph pad prism 8.0.1.244.

3. Results

3.1. Effect of the Aqueous Extract of *Monodora myristica* Seeds on the Body Weight of Rats in the Paracetamol-Induced Liver Failure

The weight of the normal control, Group 1 was 117.2 g on week 1 and 141.2 g on week 4. The weight of the negative control, Group 2 decreased significantly (157.2 vs 141.2 g; $p < 0.01$) on week 4 compared to normal control, Group 1. Treatment on week 4 with aqueous extract 200 and 300 mg/kg significantly ($p < 0.05$) increased body weight (152.2 and 154.0 g vs 141.2 g, respectively) compared to negative control, Group 2 (**Figure 1**).



The curves represent means $\pm$ ESM of 5 rats per group. * $p < 0.05$, significantly different compared to normal control. $\lambda p < 0.05$ significantly different compared to negative control. AE = Aqueous Extract.

Figure 1. Effect of the aqueous extract of *Monodora myristica* seeds on the body weight of rats in the paracetamol-induced liver failure.

3.2. Effect of the Aqueous Extract of *Monodora myristica* Seeds on Enzyme Parameters in Paracetamol Exposed Wistar Rats

The level of serum's ALT of the normal control, Group 1 was 112.4 Units/L. Pa-

racetamol in negative control Group 2 significantly increases the level of the serum's ALT (145.8 vs 112.4 Units/L; $p < 0.05$) compared to normal control, Group 1. Administration of the aqueous extract of *M. myristica* seeds, at the doses of 300 mg/kg (Group 5) significantly ($p < 0.05$) decreased the level of serum's ALT (114.4 vs 145.8 Units/L) compared to the negative control, Group 2.

The paracetamol in negative control, Group 2 significantly increases the level of the serum's AST (118.8 vs 145.8 Units/L; $p < 0.05$) compared to normal control, Group 1. Treatment with aqueous extract at the doses of 100, 200 and 300 mg/kg significantly decreased ($p < 0.05$) AST (128.4, 128.6 and 122.0 vs 145.8 Units/L respectively) compared to negative control.

Administration of the aqueous extract of *M. myristica* seeds, at the doses of 200 and 300 mg/kg markedly decreased ($p < 0.05$) ALP level (34.80 and 34.20 vs 40.00 Units/L respectively) compared to negative control.

The paracetamol significantly elevated ($p < 0.05$) the activity of T. BIL (1.80 vs 6.60 Units/L) compared to normal control. Treatment with aqueous extract at the dose of 300 mg/kg significantly decreased ($p < 0.05$) T. BIL activity (2.20 vs 6.60 Units/L) compared to negative control (**Table 1**).

Table 1. Effect aqueous extract of *Monodora myristica* seeds on AST, ALT, ALP and T. Bil in the serum of paracetamol exposed Wistar rats.

Experimental groups	ALT (Units/L)	AST (Units/L)	ALP (Units/L)	T. BIL (Units/L)
Group 1 (Normal control, Distilled water 5 mL/kg)	112.4 ± 3.40	118.8 ± 2.20	34.50 ± 2.10	1.80 ± 0.80
Group 2 (Negative control, Paracetamol 2 g/kg)	145.8 ± 3.40*	145.8 ± 3.10*	40.00 ± 1.50*	6.60 ± 0.30*
Group 3 (Positive control, Silymarine 25 mg/kg)	142.2 ± 3.60	131.8 ± 4.00 ¹	39.60 ± 1.40	2.20 ± 0.40 ¹
Group 4 (Paracetamol 2 g/kg + AE 100 mg/kg)	141.8 ± 4.00	128.4 ± 2.60 ¹	36.80 ± 2.20	4.80 ± 0.80
Group 5 (Paracetamol 2 g/kg + AE 200 mg/kg)	132.4 ± 3.40	128.6 ± 2.40 ¹	34.80 ± 1.40 ¹	2.60 ± 0.02
Group 6 (Paracetamol 2 g/kg + AE 300 mg/kg)	114.4 ± 1.40 ¹	122.0 ± 3.00 ^{1,1}	34.20 ± 2.80 ¹	2.20 ± 0.40 ¹

The values are expressed as mean ± ESM; n = 5. * $p < 0.05$, significantly different compared to Normal control; ¹ $p < 0.05$ and ^{1,1} $p < 0.01$ significantly different compared to negative control. AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALP = Alkaline phosphatase; T. BIL = Total bilirubin, AE = Aqueous extract.

3.3. Effect of the Aqueous Extract of *Monodora myristica* Seeds on Lipid Parameters of Liver in Paracetamol-Induced Liver Injury

The paracetamol significantly increased ($p < 0.05$) serum total cholesterol levels in negative control, Group 2 (41.20 vs 58.80 mg/dL) compared to the normal control Group 1. Treatment of the rats with *M. myristica* seeds at the dose of 300 mg/kg (Group 5) significantly decreases ($p < 0.05$) the serum's total cholesterol activity (58.80 vs 45.60 mg/dL) compared to the negative group.

The paracetamol significantly elevated the activity of triglyceride level in negative control (53.20 vs 74.40 mg/dL) compared to normal control ($p < 0.01$). Treatment of the rats with the three different doses 100, 200 and 300 mg/kg of the aqueous extract of *M. myristica* seeds significantly ($p < 0.01$) decreases the activity of

triglyceride level (63.00, 62.20 and 60.40 mg/dL, respectively) compared to negative control.

The paracetamol significantly reduces the activity of HDL-Cholesterol (31.60 vs 19.00 mg/dL) compared to normal control ($p < 0.01$). Administration of the rats with the doses 200 and 300 mg/kg of the aqueous extract of *M. myristica* seeds significantly increases the level of HDL-Cholesterol (26.00 and 26.40 vs 19.00 mg/dL) when compared to the negative control ($p < 0.05$).

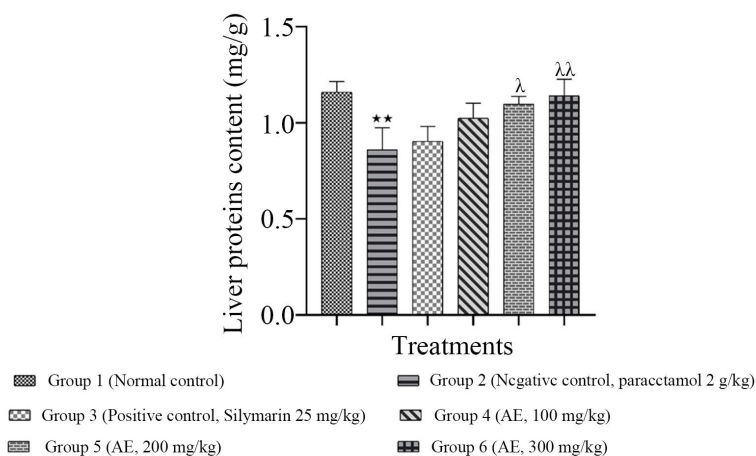
Administration of the rats with the doses of 100, 200 and 300 mg/kg does not show any significant change in the level of LDL-Cholesterol (**Table 2**).

Table 2. Effect of aqueous extract of *M. myristica* seeds on total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol in the serum of paracetamol exposed wistar rats.

Experimental groups	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)
Group 1 (Normal control, Distilled water 5 mL/kg)	41.20 $\pm$ 1.60	40.20 $\pm$ 1.20	31.60 $\pm$ 1.40	1.56 $\pm$ 1.20
Group 2 (Negative control, Paracetamol 2 g/kg)	58.80 $\pm$ 1.40*	74.40 $\pm$ 1.10**	19.00 $\pm$ 1.60**	24.92 $\pm$ 1.10**
Group 3 (Positive control, Silymarine 25 mg/kg)	57.60 $\pm$ 1.20	51.80 $\pm$ 2.60 ^λ	24.40 $\pm$ 1.40	22.84 $\pm$ 1.00
Group 4 (Paracetamol 2 g/kg + AE 100 mg/kg)	56.20 $\pm$ 2.60	63.00 $\pm$ 1.40 ^λ	22.60 $\pm$ 2.01	21.10 $\pm$ 1.30
Group 5 (Paracetamol 2 g/kg + AE 200 mg/kg)	50.20 $\pm$ 1.60	62.20 $\pm$ 2.20 ^λ	26.00 $\pm$ 1.20 ^λ	11.76 $\pm$ 2.00 ^λ
Group 6 (Paracetamol 2 g/kg + AE 300 mg/kg)	45.60 $\pm$ 1.20 ^λ	60.40 $\pm$ 2.00 ^{λλ}	26.40 $\pm$ 1.40 ^λ	7.12 $\pm$ 1.40 ^{λλ}

Values are expressed as mean $\pm$ ESM; (n = 5). * $p < 0.05$ and ** $p < 0.01$ significantly different compared to Normal control, ^λ $p < 0.05$ and ^{λλ} $p < 0.01$ significantly different compared to negative control. HDL-cholesterol = High-density lipoprotein; LDL-cholesterol = Low density lipoprotein; AE = Aqueous extract.

3.4. Effect of *Monodora myristica* Seeds Aqueous Extract on Proteins Concentration on Paracetamol Exposed Wistar Rats



The histograms represent means $\pm$ ESM of 5 rats per group. ** $p < 0.01$, significantly different compared to normal control. ^λ $p < 0.05$ and ^{λλ} $p < 0.01$, significantly different compared to negative control. AE = Aqueous Extract.

Figure 2. Effect of *M. myristica* seeds aqueous extract on proteins concentration on paracetamol exposed Wistar rats.

Treatment with paracetamol had significantly ($p < 0.01$) decreasing liver's protein activity in negative control (1.16 vs 0.86 mg/g) compared to the normal control. Co-administration of rats with paracetamol and the plant extract of 200 mg/kg and 300 mg/kg doses normalized the protein activity by significantly increasing the protein concentration (1.09 and 1.14 vs 0.86 mg/g; $p < 0.05$ and $p < 0.01$ respectively) when compared to negative control (**Figure 2**).

3.5. Effect of the Aqueous Extract of *Monodora myristica* Seeds on Biochemical Oxidative Stress Markers of the Liver on Paracetamol Exposed Wistar Rats

The CAT activity in normal control was 18.80 IU/mg of proteins. The paracetamol alone significantly ($p < 0.01$) decreased CAT activity (18.80 vs 12.00 IU/mg of proteins) compared to normal control.

The dose of 300 mg/kg of plant extract significantly increased catalase activity (12.00 vs 16.74 IU/mg of proteins) compared to the negative control ($p < 0.05$).

Superoxide dismutase (SOD) activity of the liver was significantly reduced by treatment of rats with paracetamol alone (225 vs 129 IU/mg of proteins) compared to the normal control ($p < 0.05$). Co-treatment of rats with paracetamol and the doses of 100, 200 and 300 mg/kg of *M. myristica* seeds increased significantly the activity of SOD (232, 239 and 247 vs 129 IU/mg of proteins; $p < 0.05$, $p < 0.05$ and $p < 0.01$ respectively), as compared to the negative control. Malondialdehyde (MDA) did not show significant difference with aqueous extract (**Table 3**).

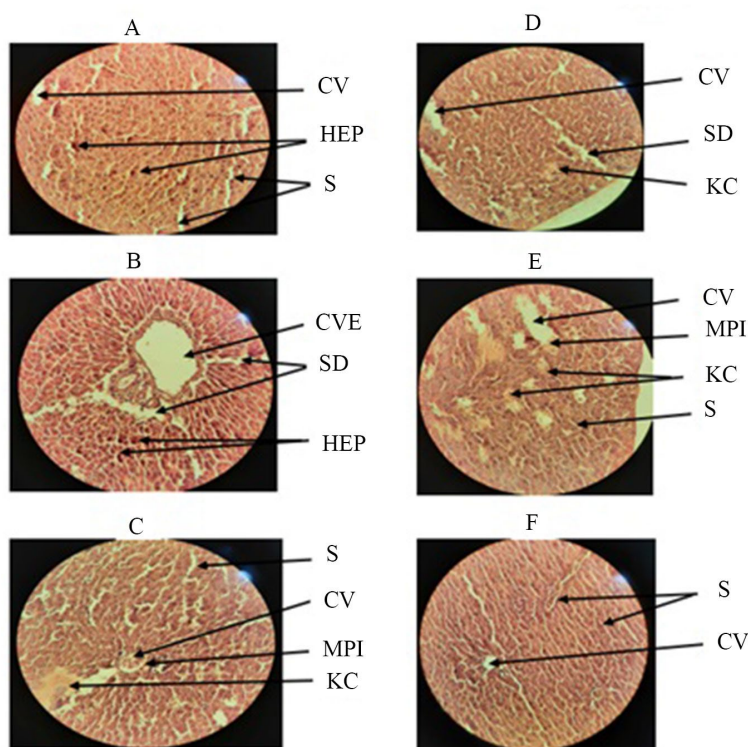
Table 3. Effect of the aqueous extract of *Monodora myristica* seeds on biochemical oxidative stress markers of the liver of paracetamol exposed wistar rats.

Experimental groups	Stress parameters		
	CAT activity (IU/mg of proteins)	MDA activity (IU/mg of proteins)	SOD (IU/mg of proteins)
Group 1 (Normal control, Distilled water 5 mL/kg)	18.80 ± 0.73	0.76 ± 0.01	225 ± 1.52
Group 2 (Negative control, Paracetamol 2 g/kg)	12.00 ± 0.69**	1.38 ± 0.01*	129 ± 0.82*
Group 3 (Positive control, Silymarine 25 mg/kg)	14.60 ± 0.16	0.64 ± 0.03 ^λ	205 ± 0.82 ^{λλ}
Group 4 (Paracetamol 2 g/kg + AE 100 mg/kg)	13.00 ± 0.07	1.28 ± 0.01	232 ± 1.34 ^λ
Group 5 (Paracetamol 2 g/kg + AE 200 mg/kg)	13.80 ± 0.21	1.22 ± 0.01	239 ± 2.59 ^λ
Group 6 (Paracetamol 2 g/kg + AE 300 mg/kg)	16.74 ± 0.14 ^λ	1.14 ± 0.01 ^λ	247 ± 1.31 ^λ

Values are expressed as mean ± ESM; n = 5. * $p < 0.05$ and ** $p < 0.01$ significantly different compared to Normal control. ^λ $p < 0.05$ and ^{λλ} $p < 0.01$ significantly different compared to negative control. CAT = Catalase, SOD = Superoxide dismutase; MDA = Malondialdehyde; AE = Aqueous extract.

3.6. Effects of Aqueous Extract of *M. myristica* Seeds on the Paracetamol Induced Damage of the Liver's Microstructure

Histological findings supported the results obtain from biochemical estimation. As shown in **Figure 3**, the liver sections of normal group (**Figure 3(A)**) showed normal hepatic parenchyma architecture with hepatocytes arranged in cobblestones around the central lobular vein and also regular liver histology. Whereas, negative group (**Figure 3(B)**) showed liver alterations characterized by degenerating cells or hepatocellular confluent necrosis, mild portal inflammation or bile plugs or obstruction of bile ducts, and sinusoid dilation. The rats treated with the highest dose of aqueous extract fruits (300 mg/kg) of *M. myristica* (**Figure 3(F)**) showed an improvement in liver structures.



Stained with hematoxylin and eosin. Magnification ($\times 400$). Normal control (**Figure 1(A)**), Negative control (**Figure 3(B)**), positive control (**Figure 3(C)**), AE 100 mg/kg (**Figure 3(D)**), AE 200 mg/kg (**Figure 3(E)**), AE 300 mg/kg (**Figure 3(F)**). CV = Central vein; HEP = Hepatocytes; S = Sinusoids; DC = Degenerating cells; MPI = Mild portal inflammation; KC = Kupffer cells; CVE = Central vein enlargement; SD = Sinusoid dilation.

Figure 3. Effects of aqueous extract of *M. myristica* seeds on the paracetamol induced damage of the liver's microstructure.

4. Discussion

The overdoses of acetaminophen are metabolized to toxic intermediate N-acetyl-p-benzoquinone imine (NAPQI) [6]. The results indicated that the body weight of negative control was significantly lower ($p < 0.01$) compared to normal control

suggesting that NAPQI depletes hepatic glutathione and causes hepatocellular necrosis. This liver dysfunction may impair nutrient metabolism, protein synthesis, and energy homeostasis, resulting in body weight loss. Treatment with aqueous extract 200 and 300 mg/kg significantly ($p < 0.05$) increased body weight compared to negative control, Group 2 suggesting that it could counteract the action of NAQI and restored liver function and body weight. The similar results were obtained on effects of aqueous aloe vera gel extract on acetaminophen-induced liver damage: a histomorphological, hematological and biochemical study in Albino rats [31]. These effects are driven by bioactive constituents like flavonoids, anthraquinones, and polysaccharides, which counteract NAPQI-induced damage and promote tissue repair.

Additionally, paracetamol in negative control, Group 2 significantly increases the level of the serum's ALT, AST, ALP and T.BIL compared to normal control. Elevations in serum enzyme levels are taken as the relevant indicators of liver toxicity and injuries whereas increasing in total bilirubin level is a measure of liver function. The cellular injury leads to oxidative stress, mitochondrial dysfunction, increased production of reactive oxygen species (ROS), and ultimately hepatocyte necrosis. When hepatocytes are damaged or destroyed, the intracellular enzymes ALT, AST, ALP, and T. BIL are released into the bloodstream [32]. The aqueous extract of *M. myristica* seeds, 300 mg/kg significantly ($p < 0.05$) decreased the level of serum's ALT. The doses of 100, 200 and 300 mg/kg significantly decreased ($p < 0.05$) AST. The doses of 200 and 300 mg/kg markedly decreased ($p < 0.05$) ALP level. The aqueous extract at the dose of 300 mg/kg significantly decreased ($p < 0.05$) T. BIL activity compared to negative control. This suggests that bioactive compounds in the extract act as antioxidants were able to neutralize ROS. Compounds such as flavonoids, polyphenols, and tannins are capable of preserving hepatocyte membrane integrity, thereby preventing the leakage of intracellular enzymes into the bloodstream [18]. This work is consistent with the results of some researchers who have experimented *Ocimum basilicum* leaf extract and demonstrated its mechanisms of hepato-renal protective activity effects against paracetamol toxicity in the rat model [32].

Furthermore, paracetamol in negative control significantly increased ($p < 0.05$) serum total cholesterol levels, activity of triglyceride level, LDL cholesterol level and decreased activity of HDL-Cholesterol and proteins compared to the normal control. When hepatocytes are damaged, fatty acid beta-oxidation decreases. On the other hand, the liver's capacity to clear cholesterol-rich lipoproteins is reduced promoting triglyceride synthesis [33]. It is known that the liver is the central organ for lipoprotein metabolism, its destruction leads to a decrease in the level of apolipoprotein (ApoA-I), the primary protein associated with HDL cholesterol. Thus, NAQI-induced liver damage reduces ApoA-I synthesis. Concurrently, NAQI causes hepatocellular necrosis, thereby diminishing the liver's capacity to synthesize proteins [24]. Treatment of the rats with *M. myrista* seeds at the dose of 300 mg/kg (Group 5) significantly decreases ($p < 0.05$) the serum's total cholesterol

activity compared to negative control. Additionally, the three different doses 100, 200 and 300 mg/kg of the aqueous extract of *M. myrista* seeds significantly ($p < 0.01$) decrease the activity of triglyceride level and the doses 200 and 300 mg/kg of the aqueous extract of *M. myrista* seeds significantly increase the level of HDL-Cholesterol compared to negative control. The plant extract of 200 mg/kg and 300 mg/kg doses significantly increased the protein concentration ($p < 0.05$ and $p < 0.01$ respectively). The observed effects could be linked to the presence of bioactive compounds in the aqueous extract. The flavonoids promote the conversion of cholesterol into bile acids, primarily by stimulating the activity of the hepatic enzyme cholesterol 7 α -hydroxylase (CYP7A1). Polyphenols increase CYP7A1 expression [19]. The tannins present reduce liver damage and enable the liver to improve lipid metabolism of hepatic injury [34]. This work aligns with the findings of researchers who tested flavonoids and saponins extracted from black bean (*Phaseolus vulgaris* L.) seed coats on mice and demonstrated that they modulate lipid metabolism and biliary cholesterol secretion [34].

Silymarin was used as a reference drug due to its hepatoprotective properties [14]. However, its effect did not result in the complete normalization of the hepatic parameters (ALT and ALP) and lipid parameters (total cholesterol, HDL cholesterol, and LDL cholesterol) evaluated. The values of these markers which remained statistically insignificant compared to those observed in the negative control group suggest that the recovery of liver function was only partial. This limited response may be attributed to the persistence of liver damage observed in histological sections, the duration of treatment, and the fact that various biochemical and lipid parameters do not necessarily recover at the same rate following liver injury. Consequently, while our results with silymarin show a significant improvement in certain indicators of liver damage (AST and total bilirubin) and the lipid profile (triglycerides), they do not support the conclusion that normal liver function was fully restored.

On the other hand, research has shown that the excessive formation of the toxic metabolite NAPQI from paracetamol depletes glutathione and leads to a reduction in cellular reserves. Excessive production of reactive oxygen species (ROS) increases free radicals such as the superoxide anion and hydrogen peroxide thereby causing a decrease in SOD and CAT levels. ROS attack the polyunsaturated fatty acids of cell membranes, triggering lipid peroxidation, which results in the production of MDA [35]. The dose of 300 mg/kg of plant extract significantly increased catalase (CAT) activity ($p < 0.05$). The doses of 100, 200 and 300 mg/kg of *M. myrista* seeds markedly increased the activity of superoxyde dismutase (SOD) compared to the negative control. This suggests that the bioactive compounds influenced oxidative stress parameters. Alkaloids exhibit antioxidant properties and limit NAPQI-induced damage. Terpenoids and triterpenes protect mitochondria and reduce ROS formation, thereby increasing SOD and CAT activities [18]. Similar results were obtained by researchers investigating aqueous aloe vera gel extract, who demonstrated that its constituents increase SOD and CAT activities in paracetamol-intoxicated rats [31].

In addition, histological observations (**Figure 3**) have shown that oral administration of overdose of paracetamol (2 g/kg) induced hepatocyte degeneration, kuffer cells, sinusoidal dilation and mild portal inflammation in negative group (**Figure 3(B)**) when compared to normal group (**Figure 3(A)**). These results indicate that overdose of paracetamol inducing intoxication of liver might cause hepatocyte destruction which could explain the observed liver abnormalities [31]. The dose of 300 mg/kg of aqueous extract seeds of *M. myristica* repairs damaged hepatocytes, suggesting that the aqueous extract seeds of *M. myristica* might contain components capable of regenerating and/or repairing hepatocytes.

5. Conclusion

The findings of the present assessment demonstrated that aqueous extract of seeds of *M. myristica* is beneficial for patients with hepatic diseases. Aqueous extract of seeds of *M. myristica* decreased serum levels (ALT and AST) transaminases, ALP, total bilirubin, triglyceride, HDL-cholesterol, LDL-cholesterol and total cholesterol. It also decreased CAT and SOD. Furthermore, it has antioxidant and therapeutic effects. Histological examination showed that the highest doses of 300 mg/kg of *Monodora myristica* extract attenuated the hepatic alterations, with clearly defined nuclei and cell borders.

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Consent for Publication

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Author Contributions

Oumar Mahamat, Ndikum Cynthia Mata and Youmbie Djanche Duplex Bonheur conceived and designed the study. Ndikum Cynthia Mata and Youmbie Djanche Duplex Bonheur performed the experiments. Ndikum Cynthia Mata and Youmbie Djanche Duplex Bonheur analysed and interpreted the data. Kada Sanda Antoine and Youmbie Djanche Duplex Bonheur contributed to the writing of the manuscript. All authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publica-

tion of this paper.

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List of Abbreviation

ALT: Alanine aminotransferase
 AST: Aspartate aminotransferase
 SOD: Superoxide dismutase
 ALP: Alkaline phosphatase
 MDA: Malondialdehyde
 NAPQI: N-acetyl-benzoquinone imine
 CYP7A1: Cholesterol 7 α -hydroxylase