

Original Article

Treatment with *Markhamia tomentosa* Benth. K. Schum prevents carbon tetrachloride-induced liver damage in rats

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ABSTRACT

Objective: *Markhamia tomentosa* (Bignoniaceae) is a medicinal plant with several pharmacological properties. However, its hepatoprotective effects have been little studied. The aim of this study was to evaluate the protective effects of the aqueous trunk bark extract of this plant against carbon tetrachloride (CCl₄)-induced liver injury in rat.

Material and Methods: Thirty male albino Wistar rats were divided into six groups (five each) with Groups 1, 2, 3, and 4 as negative (distilled water), normal (distilled water), positive (silymarin 25 mg/kg), and plant extract (50 mg/kg) controls, respectively. Groups 5 and 6 were used as test groups and were given plant extract (25 or 50 mg/kg, respectively). Rats were pretreated once a day for 14 days orally with different substances. CCl₄ (0.5 mL/kg, i.p.) was administered on days 4 and 11 to all groups except Groups 1 and 4, to induce hepatitis. The rats were then sacrificed on day 15; liver functions and oxidative stress were assessed as well as histopathological changes.

Results: *M. tomentosa* extract significantly and dose dependently decreased alanine aminotransferase, aspartate aminotransferase, gamma-glutamine aminotransferase, total bilirubin, total cholesterol, and malondialdehyde values while increasing catalase, and glutathione values compared to the CCl₄-treated group. Histological findings showed a reduction in necrosis and inflammatory cell infiltration in the liver while the lumen of distal and proximal tubes was improved in the kidney by the plant extract. These results may be due to some of the major bioactives compounds found in the aqueous extract.

Conclusion: These findings suggest that the aqueous extract of *M. tomentosa* may have liver protective effects through its antioxidant and anti-inflammatory mechanisms, supporting thereby its ethnomedicinal uses.

Keywords: Liver diseases, *Markhamia tomentosa*, Hepatoprotective activity, Carbone tetrachloride, Antioxidant effect

INTRODUCTION

The liver is a major organ involved in the metabolism of endogenous or exogenous substances.^[1,2] This metabolic activity exposes the liver cells to the toxicity of xenobiotic and their metabolites and thereby increases the risk of causing injury to liver, leading to liver inflammation. Hepatitis, an inflammation of the liver tissue, can also be caused by infectious agents (bacterial, virus, and parasites) or toxins that damage the liver cells.^[3,4] During the process of liver injury, intracellular substances leak out of injured cells, leading to the activation of neutrophils and monocytes, and massive recruitment of these immune cells into the damaged liver tissue. Inflammatory cells are known to actively promote the production of reactive species, tumor necrosis factor-alpha

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(TNF- α), and other pro-inflammatory cytokines that are involved in the worsening of tissue lesions.^[5]

Carbon tetrachloride (CCl₄) is one of the chemical products frequently used to induce liver injury.^[6] The toxicity of CCl₄ results from its metabolism by cytochrome P450 in the highly reactive species, trichloromethyl radical (CCl₃), and trichloromethyl peroxy radical (CCl₃OO).^[7] These molecules attack polyunsaturated fatty acids of biological membranes and cause lipid peroxidation, which contributes to severe cellular damages.^[8,9] Destruction of mitochondria membrane by CCl₄ metabolites triggers caspase-3-dependent apoptosis through specific cleavage of key cellular proteins by caspase-3, a major cell death proteases.^[10] It has been proposed that the primary way to protect against CCl₄-induced liver damage is to inhibit the production of free radicals.^[11]

Medicinal plants have been used for centuries to treat diseases due to their safety and efficacy, as well as their richness in substances that could be used for therapeutic purposes or as precursors to the synthesis of useful drugs.^[12] In this adventure, no part of the plant is left unexplored. Stem, roots, leaves, flowers, fruits, bark, and wood are all studied for their medicinal potential.

Markhamia tomentosa is a medicinal plant of the Bignoniaceae family found mainly in tropical regions.^[13] Ethnopharmacological data indicate its use against headaches, canker, lumbago, chest pain, edema, rheumatic pain, scrotal elephantiasis, anemia, diarrhea, backache, sore eyes, intercostal pain, snakebite/venom, ailments of the reproductive system, lung disorders, gout, bouts of swamp fever, and external skin diseases.^[14-17] A study revealed the presence of alkaloids, tannins, saponins, anthraquinones, cardiac glycosides, glycosides, phenols, and flavonoids in *M. tomentosa* methanol leaf extract and two compounds, namely, 2-acetyl-6-methoxynaphtho[2,3-b]furan-4,9-dione and 2-acetylnaphtho[2,3-b]furan-4,9-dione were identified in the ethyl acetate leaf extract.^[18,19] Based on these usages and probably on phytochemical composition, the previous studies demonstrated that *M. tomentosa* possessed antiprotozoal and larvicidal activities, analgesic effects, acute and chronic anti-inflammatory effects, and antiarthritic effects.^[18-22] It was also proved that aqueous and methanol leaf extract of *M. tomentosa* was relatively non-toxic to rats.^[17] More recently, it was found that the methanol leaf extract of *M. tomentosa* exhibited a protective effect on D-galactosamine/lipopolysaccharide-induced acute hepatitis.^[23] However, to the best of our knowledge, no research on the CCl₄-induced liver damage has been conducted. Furthermore, the search for a viable natural remedy for liver diseases led us to investigate the potential protective benefits of aqueous extract of *M. tomentosa* stem bark against CCl₄-induced liver injury in rats.

MATERIAL AND METHODS

Plant material

M. tomentosa bark was collected in July 2019, in the locality of Bayangam, West region-Cameroon. The identification was made by botanist of the National Herbarium of Cameroon, in comparison with an existing voucher specimen registered under the number 1974/SRFK. The harvested bark was cut into small pieces that were dried in the shade. The dried bark was crushed with an electric grinder and sieved, and the powder was carefully stored in a plastic bottle for extraction.

Preparation of the aqueous extract

The plant extract was prepared as previously described by Temdie *et al.*^[19] Briefly, 150 g of the *M. tomentosa* trunk bark powder was introduced into the adiabatic device with 750 mL of hot distilled water (60°C) and the mixture was stirred regularly for 24 h. The preparation was decanted and filtered through Whatman No. 3 paper. Two milliliters of the filtrate were evaporated in an oven to determine the extraction concentration (28 mg/mL). The remaining filtrate was stored at -20°C for later use.

Experimental animals

The study was conducted on male albino rats weighing 150 \pm 10 g. These rats were reared under natural conditions (room temperature, 12 h/12 h photoperiod, and average humidity of 79 \pm 10%) with free access to water and standard food. The research was conducted in accordance with the revised protocols for the care of laboratory animals (NIH publication No. 85-23, 1985) and the authorization granted by the Cameroon National Ethical Committee (Reg. No. FWAIRD 0001954).

Qualitative phytochemicals analysis

Qualitative phytochemical analysis of the aqueous extract of *M. tomentosa* bark was performed to look for compounds such as polyphenols, flavonoids, tannins, anthraquinones, saponins, anthocyanins, triterpenes, and alkaloids following the protocol previously described by N'guessan *et al.*^[24]

Experimental design

Thirty rats were divided into six groups of five rats each made up of four control groups (healthy, hepatitis, extract, and positive control groups) and two test groups according to treatment received [Table 1]. The animals were weighed on the 1st day before treatment and then daily. Distilled water, silymarin (Micro Labs Limited, Bengaluru, India), and plant extract at the doses of 25 or 50 mg/kg were administered orally (10 mL/kg) at a daily dose for 14 days. Liver injury was

Table 1: Distribution of the rats in the different groups in accordance with the treatment received.

Selected animals	30 rats				
Groups formed	Hepatitis control	Healthy control	Positive control	Extract control	Test groups
Treatment received	H ₂ O+CCl ₄	H ₂ O+Olive oil	Sily 25+CCl ₄	ATBE 50+Olive oil	ATBE 50+CCl ₄ ATBE 25+CCl ₄
Sily 25: Silymarin at a dose of 25 mg/kg. ATBE 50 or 25: Aqueous trunk bark extract of <i>Markhamia tomentosa</i> at the dose of 50 or 25 mg/kg.					

induced after 12 h of fasting by intraperitoneal injection of a mixture of an equal volume (1/1) of carbon tetrachloride (Sigma-Aldrich, St. Louis, Missouri, USA) and olive oil (Lesieur, 29, Quai Aulagnier 92665 Sur Seine Cedex, France) at the dose of 0.5 mL/kg, on days 4 and 11, except for the healthy and extract control groups, which received an equivalent volume of olive oil in the same manner.^[25] At the end of the treatment, the rats were sacrificed under anesthesia (ethylic ether) by decapitation. The blood sample was collected in dry tubes and centrifuged at 3000 rpm for 15 min to obtain serum. Liver and kidney were removed, rinsed in normal saline solution, and weighed. The liver and kidney sample was cold ground in Tris-HCl buffer (50 mM, pH 7.4) to obtain a homogenate (20%), which was centrifuged at 3000 rpm for 15 min. The supernatant was recovered and stored as well as the serum at -20°C for the biochemical analysis. Another sample of liver and kidney was submerged in buffered formalin (10%) for histological analysis.^[23]

Analysis of biochemical parameters

The determination of biochemical indicators such as transaminases, gamma-glutamyl-transferase, triglycerides, total protein, creatinine, total cholesterol, and total bilirubin was performed according to the kit protocols (Chronolab systems, Barcelona, Spain), revised in 2017. The analysis of superoxide dismutase, malondialdehyde, catalase, and reduced glutathione was done according to the Madoui protocols.^[26]

Histological analysis

Liver and kidney samples previously kept in buffered formalin (10%) were subjected to the following histological techniques.^[22] The samples were fixed, dehydrated, and impregnated. After impregnation, the samples were then embedded in the paraffin to form blocks. A 50 µm thick section of liver and kidney tissues was stained with hematoxylin-eosin and observed under a light microscope (ZEISS Axioskop, Paris, France).

Statistical analysis

Values are expressed as mean ± SEM. Statistical differences between groups were determined using analysis of variance

followed by Dunnett's test. Data were analyzed using GraphPad Prism (version 5.03). $P \leq 0.05$ was considered statistically significant.

RESULTS

Qualitative phytochemical composition of the extract

The phytochemical test revealed the presence of polyphenols, tannins, flavonoids, saponins, anthocyanins, and anthraquinones in the aqueous trunk bark extract [Table 2]. Alkaloids and triterpenes were not found in the aqueous trunk bark extract of *M. tomentosa*.

Body weight gain, liver and kidney absolute and relative weights

The results show normal growth of healthy animals with a weight gain in relation to the 1st day of treatment of $0.50 \pm 3.43\%$ on the 4th day and of $15.32 \pm 2.10\%$ on the 14th day [Table 3]. Treatment of animals with CCl₄ resulted in a significant loss of body mass (-1.11 ± 2.16 and $-4.74 \pm 2.91\%$), respectively, on the 4th and 14th days, compared to the healthy control. Treatment with silymarin (25 mg/kg) prevented body weight loss until the 4th day ($0.45 \pm 0.99\%$), after which weight loss was recorded until the 14th day ($-4.17 \pm 1.51\%$). Pre-treatment with the extract (25 mg/kg) significantly improved weight gain during treatment but a significant weight loss was registered at the end of the experimental period ($-2.49 \pm 3.33\%$), compared to the healthy control group. Administered alone, the extract did not impair animal growth during the study as compared to the healthy group.

Hepatitis control rats showed a slight reduction of the relative liver mass ($3.40 \pm 0.13\%$) compared to healthy animals ($3.50 \pm 0.06\%$). Silymarin treatment did not affect the relative liver mass of rats compared to different controls groups ($3.17 \pm 0.18\%$). A decrease in the relative mass of the liver was recorded at the end of the study in animals treated with the aqueous trunk bark extract of *M. tomentosa*. This reduction was significant at the dose of 50 mg/kg ($3.02 \pm 0.02\%$). A significant reduction was also observed after treatment of healthy animals with the plant extract ($3.06 \pm 0.07\%$), as compared to control groups. At the end of the treatment, no significant variation in the relative kidney weight was observed in different groups [Table 3].

Effect of the extract on enzymatic biochemical indicators of liver function

The treatment of animals with CCl₄ resulted after 14 days in a significant increase in the activity of alanine aminotransferase (795.78 ± 15.78 U/L) compared to the healthy control (189.30 ± 50.42 U/L). Treatment with silymarin (25 mg/kg) significantly reduced ALT activity to 207.21 ± 31.68 U/L. Plant trunk bark extract (25 or 50 mg/kg) also reduced in a significant manner the ALT activity at 559.39 ± 91.69 U/L or 364.65 ± 39.00 U/L, respectively, compared to the hepatitis control [Table 4].

At the end of treatment, CCl₄ caused a significant increase in aspartate aminotransferase activity (487.08 ± 57.92 U/L) compared to the healthy control (273.11 ± 36.76 U/L). Treatment with silymarin resulted in a significant reduction in AST activity (297.35 ± 20.89 U/L) compared to the hepatitis control [Table 4]. The extract (25 or 50 mg/kg) weakly decreased AST activity to 396.47±66.01 U/L or 353.79 ± 41.57 U/L, respectively, compared to healthy control.

A significant increase in the level of gamma-GT (46.16 ± 4.32 U/L) was recorded on the 14th day, in animals after induction of hepatitis with CCl₄ as compared to the healthy control (17.59 ± 3.26 U/L). The treatment with

silymarin significantly reduced the level of gamma-GT (23.60 ± 2.87 U/L); likewise, the rats treated with the plant extract (25 or 50 mg/kg) had a significantly decreased gamma-GT activity (21.74 ± 4.69 or 15.42 ± 1.48 U/L, respectively), compared to the hepatitis control [Table 4]. *M. tomentosa* trunk bark aqueous extract did not affect the activity of enzymatic biochemical parameters of the liver function of healthy animals (24.00 ± 1.88 U/L) as compared to healthy control.

Effect of the extract on non-enzymatic biochemical indicators of liver and renal functions

Induction of hepatitis by CCl₄ injection caused 2 weeks after its administration, a significant increase in the concentration of total bilirubin (0.64 ± 0.09 mg/dL) compared to the healthy control (0.31 ± 0.05 mg/dL). Treatment of animals with silymarin significantly prevented the elevation of total bilirubin concentration (0.27 ± 0.04 mg/dL) compared to the hepatitis control [Table 5]. Administration of the extract (25 or 50 mg/kg) significantly reduced the level of total bilirubin (0.29 ± 0.12 or 0.23 ± 0.00 mg/dL, respectively) compared to the hepatitis control.

No significant increase in creatinine level was observed between the healthy control (0.52 ± 0.01 mg/dL) and hepatitis control (0.65 ± 0.06 mg/dL). Silymarin (25 mg/kg) significantly reduced creatinine levels (0.46 ± 0.01 mg/kg) compared to the hepatitis control. The extract (25 or 50 mg/kg) did not significantly influence plasma creatinine concentration (0.53 ± 0.04 or 0.56 ± 0.04 mg/dL, respectively), compared to the hepatitis control [Table 5]. In the extract control group (50 mg/kg), a significant reduction of creatinine level (0.47 ± 0.01 mg/kg) was observed, compared to the hepatitis control.

The healthy control (5.33 ± 0.64 mg/dL) and hepatitis control (6.20 ± 0.51 mg/dL) show no non-significant variation in the plasmatic level of total proteins, for in the case in rats treated with the silymarin (25 mg/kg), there is a significant reduction in the level of total proteins (3.40 ± 0.63 mg/dL) compared

Table 2: Phytochemical composition of the aqueous extract of the bark of *Markhamia tomentosa*.

Compounds found	Observations
Polyphenols	+
Tannins	+
Flavonoids	+
Anthraquinones	+
Alkaloids	-
Triterpenes	-
Saponins	+
Anthocyanins	+

+: Present, -: Absent

Table 3: Changes in the body, liver and kidney weights of healthy and CCl₄-treated rats under *Markhamia tomentosa* aqueous extract treatment.

Treatment	Body weight gain (%)			Absolute weight (g)		Relative mass (%)	
	Day 4 th	Day 8 th	Day 14 th	Liver	Kidney	Liver	Kidney
H ₂ O+CCl ₄	-1.11±2.16 [#]	-1.40±2.30	-4.74±2.91 [#]	4.69±0.32	0.97±0.05	3.40±0.13	0.71±0.04
H ₂ O+Olive oil	0.50±3.43*	0.33±4.09	15.32±2.10*	4.90±0.15	0.96±0.05	3.50±0.06	0.68±0.03
Sily 25+CCl ₄	0.45±0.99*	-2.49±0.59	-4.17±1.51 [#]	4.05±0.20 [#]	0.88±0.05	3.17±0.18	0.68±0.03
ATBE 50+ Olive oil	3.25±0.12*	8.63±1.11 ^{#*}	20.99±1.18*	5.02±0.15	1.01±0.02	3.06±0.07 ^{***}	0.61±0.01
ATBE 50+CCl ₄	4.68±0.74*	4.05±0.31*	-2.49±3.33 [#]	4.14±0.05	0.95±0.02	3.02±0.02 ^{***}	0.69±0.01
ATBE 25+CCl ₄	5.11±2.43*	1.74±2.85	5.91±4.54 [#]	4.66±0.37	0.90±0.06	3.20±0.14	0.62±0.00

Each value represents the mean±SEM. n=5 number of animals in each group. *P<0.05 compared to hepatitis control group. [#]P<0.05 compared to the healthy control group. Sily 25: Silymarin at a dose of 25 mg/kg. ATBE 50 or 25: Aqueous trunk bark extract of *Markhamia tomentosa* at the dose of 50 or 25 mg/kg, CCl₄: Carbon tetrachloride.

to hepatitis control. The rats treated with the extract (25 or 50 mg/kg) as well as the extract control group show an increase in the level of total proteins with a significant effect in the animals having received only the plant extract (9.27 ± 0.28 mg/dL) compared to the healthy and hepatitis controls.

No significant variation in triglyceride level was observed in the different groups compared to the healthy and hepatitis control.

The administration of CCl₄ induced a significant increase in the total cholesterol level (80.72 ± 4.53 mmol/L) compared to the healthy control (52.31 ± 6.38 mmol/L). Treatment with the extract (25 or 50 mg/kg) and silymarin (25 mg) did not significantly reduce total cholesterol levels (79.72 ± 7.95 ; 75.37 ± 4.66 ; or 61.29 ± 5.93 mmol/L, respectively) compared to the hepatitis control. Taken alone, *M. tomentosa* extract induced a significant increase in cholesterol levels (75.27 ± 0.84 mmol/L) compared to that of the healthy control.

Table 4: Fluctuations in enzymatic biochemical parameters of the liver function of healthy and CCl₄-treated rats under *Markhamia tomentosa* aqueous extract treatment.

Treatments	ALT (U/L)	AST (U/L)	γ -GT (U/L)
H ₂ O+CCl ₄	795.78±15.78**	487.08±57.92**	46.16±4.32**
H ₂ O+Olive oil	189.30±50.42**	273.11±36.76**	17.59±3.26**
Sily 25+CCl ₄	207.21±31.68**	297.35±20.89*	23.60±2.87**
ATBE	219.04±31.54**	301.70±18.60*	24.00±1.88**
50+Olive oil			
ATBE	364.65±39.00**	353.79±41.57	15.42±1.48**
50+CCl ₄			
ATBE	559.39±91.69***	396.47±66.01	21.74±4.69**
25+CCl ₄			

Each value represents the mean±SEM. n=5 number of animals in each group. *P<0.05, **P<0.01, compared to the hepatitis control group, ***P<0.01 compared to the healthy control group. Sily 25: Silymarin at a dose of 25 mg/kg. ATBE 50 or 25: Aqueous trunk bark extract of *Markhamia tomentosa* at the dose of 50 or 25 mg/kg, CCl₄: Carbon tetrachloride.

Effect of the extract on hepatic and renal oxidative stress

CCl₄ induced in animals a significant increase in the level of hepatic malondialdehyde (3.13 ± 0.01 nmol/mg of protein) compared to the healthy control (2.48 ± 0.01 nmol/mg of protein). Treatment with silymarin (25 mg/kg) led to a significant reduction in the level of malondialdehyde (2.36 ± 0.06 nmol/mg of protein). The treatment with an aqueous extract (25 or 50 mg/kg) resulted in a significant drop in the level of malondialdehyde (2.67 ± 0.03 or 2.79 ± 0.04 nmol/mg of protein, respectively) compared to the hepatitis control [Table 6]. The extract control animals have a significantly lower level of malondialdehyde (1.65 ± 0.00 nmol/mg of protein) than the healthy control. CCl₄ caused a significant increase in the level of malondialdehyde in the kidney (2.75 ± 0.02 nmol/mg of protein) compared to the healthy control (1.23 ± 0.00 nmol/mg of protein). *M. tomentosa* extract (25 or 50 mg/kg) significantly reduced malondialdehyde level to 1.65 ± 0.00 or 1.66 ± 0.05 nmol/mg of protein, respectively, compared to the hepatitis control.

The results show a significant increase in catalase activity (100.40 ± 5.23 nmol/min/mg of protein) compared to the healthy control (77.19 ± 5.10 nmol/min/mg of protein). The extract at a dose of 25 or 50 mg/kg induced a significant rise in catalase activity (136.08 ± 12.98 or 155.32 ± 12.82 nmol/min/mg of protein) compared to the hepatitis control. The extract induced a significant elevation of hepatic catalase activity of the extract control group (167.30 ± 6.26 nmol/min/mg of protein), compared to the healthy and hepatitis control groups. In the kidney, the extract (25 or 50 mg/kg) caused a significant increase (141.85 ± 16.12 or 160.13 ± 9.51 , nmol/min/mg of protein, respectively) in catalase activity compared to the hepatitis control (81.26 ± 9.53 nmol/min/mg of protein). Treatment of healthy animals with the aqueous extract of *M. tomentosa* result in significant rise in renal catalase activity (150.99 ± 11.73 nmol/min/mg of protein) compared to control groups.

Table 5: Variations of non-enzymatic biochemical parameters of liver and renal functions of healthy and CCl₄-treated rats under *Markhamia tomentosa* aqueous extract treatment.

Treatments	Total bilirubin (mg/dL)	Creatinine (mg/dL)	Total protein (mg/dL)	Triglycerides (mg/dL)	Total cholesterol (mmol/L)
H ₂ O+CCl ₄	0.64±0.09 [#]	0.65±0.06	6.20±0.51	166.26±9.65	80.72±4.53**
H ₂ O+Olive oil	0.32±0.05*	0.52±0.01	5.33±0.64	130.09±11.60	52.31±6.38**
Sily 25+CCl ₄	0.27±0.04**	0.46±0.01**	3.40±0.63**	148.48±15.40	61.29±5.93
ATBE 50+Olive oil	0.35±0.05*	0.47±0.01*	9.27±0.28***	151.67±32.34	75.27±0.84 [#]
ATBE 50+CCl ₄	0.23±0.00**	0.56±0.04	6.68±0.12	116.86±14.13	75.37±4.66 [#]
ATBE 25+CCl ₄	0.29±0.12*	0.53±0.04	6.65±0.72	149.87±19.73	79.72±7.95**

Each value represents the mean±SEM. n=5 number of animals in each group. *P<0.05, **P<0.01 compared to hepatitis control group; [#]P<0.05, ^{**}P<0.01 compared to healthy control group. Sily 25: Silymarin at a dose of 25 mg/kg. ATBE 50 or 25: Aqueous trunk bark extract of *Markhamia tomentosa* at the dose of 50 or 25 mg/kg, CCl₄: Carbon tetrachloride.

Table 6: Changes in hepatic and renal oxidative stress indicators of healthy and CCl₄-treated rats under *Markhamia tomentosa* aqueous extract treatment.

Tissue	Treatment	MDA	Catalase	SOD	Glutathione
		(nmol/mg of protein)	(nmol/min/mg of protein)	(U/mg of protein)	(nmol/mg of protein)
Liver	H ₂ O+CCl ₄	3.13±0.01 [#]	100.40±5.23 [#]	23.66±2.91 [#]	267.64±8.25 [#]
	H ₂ O+Olive oil	2.48±0.01 ^{**}	77.19±5.10 [*]	49.16±0.26 ^{**}	369.09±5.26 ^{**}
	Sily25+CCl ₄	2.36±0.06 ^{**}	108.67±17.10 [#]	30.54±5.77 [#]	346.81±6.32 [*]
	ATBE50+Olive oil	1.65±0.00 ^{****}	167.30±6.26 ^{****}	33.37±7.79	390.29±29.35 ^{**}
	ATBE50+CCl ₄	2.79±0.04 ^{****}	155.32±12.82 ^{****}	28.52±6.08 [#]	391.82±30.61 ^{**}
	ATBE25+CCl ₄	2.67±0.03 ^{****}	136.08±12.98 [#]	26.66±2.51 [#]	241.47±9.17 [#]
Kidney	H ₂ O+CCl ₄	2.75±0.02 [#]	81.26±9.53	24.5±5.11 [#]	156.76±13.68
	H ₂ O+Olive oil	1.23±0.00 ^{**}	67.32±7.71	42.25±6.07 [*]	226.76±12.21
	Sily25+CCl ₄	1.33±0.00 ^{**}	74.05±18.87	38.29±2.83	190.58±1.44
	ATBE50+Olive oil	1.63±0.03 ^{****}	150.99±11.73 ^{****}	36.37±2.52	207.94±31.60
	ATBE50+CCl ₄	1.66±0.05 ^{****}	160.13±9.51 ^{****}	36.25±9.93	340.29±30.61 ^{****}
	ATBE25+CCl ₄	1.65±0.00 ^{****}	141.85±16.12 ^{****}	37.08±1.14	310.88±19.97 ^{****}

Each value represents the mean±SEM. n=5 number of animals in each group. *P<0.05, **P<0.01 compared to hepatitis control group; [#]P<0.05, ^{**}P<0.01 compared to healthy control group. Sily 25: Silymarin at a dose of 25 mg/kg. ATBE 50 or 25: Aqueous trunk bark extract of *Markhamia tomentosa* at the dose of 50 or 25 mg/kg. CCl₄: Carbon tetrachloride.

Induction of hepatitis by CCl₄ caused a significant reduction in the activity of superoxide dismutase in the liver tissue (23.66 ± 2.91 U/mg of protein) compared to the healthy control (49.16 ± 0.26 U/mg of protein). Silymarin (25 mg/kg) did not significantly prevent (30.54 ± 5.77 U/mg of protein) the reduction in superoxide dismutase activity compared to the healthy control. Similarly, the extract (25 or 50 mg/kg) did not rise significantly (26.66 ± 2.51 or 28.52 ± 6.08 U/mg of protein) the activity of superoxide dismutase compared to the healthy control [Table 6].

Hepatitis due to CCl₄ caused a significant reduction in glutathione (267.64 ± 8.25 nmol/mg of protein) compared to the healthy control (369.09±5.26 nmol/mg of protein). Administration of silymarin (25 mg/kg) significantly raised glutathione levels in rats (346.81 ± 6.32 nmol/mg of protein). The extract at a dose of 50 mg/kg caused a significant increase in the glutathione level (391.82 ± 30.61 nmol/mg of protein) compared to the hepatitis control. An increase in the glutathione level is noted in the extract control group (390.29 ± 29.35 nmol/mg of protein) compared to the hepatitis control [Table 6].

Effect of extract on CCl₄-induced liver inflammation

Microphotography of the liver of a healthy rat shows normal and uniformly stained hepatocytes with one or two nuclei. Cells are well-organized in travel and the portal space is clearly observable [Figure 1a]. The microphotography of the liver of the CCl₄-treated rat shows a remarkable invasion of the portal space and the whole hepatic parenchyma by mononuclear inflammatory leukocyte cells. Edema characterized by dilated capillaries could be found in the parenchyma where hepatocytes present dark nuclei,

indicating chromatin condensation probably as a result of a reduction in cellular activity due to inflammation [Figure 1b]. The liver section also shows cells necrosis represented by a pile of cells nuclei without a plasma membrane surrounding each. When the rats were treated with silymarin (25 mg/kg) or aqueous extract (50 or 25 mg/kg), a clear improvement of the histological parameters was observed compared to the control and mononuclear inflammatory leukocyte infiltration was also reduced [Figures 1c-f].

Effect of extract on CCl₄-induced kidney inflammation

Microphotography of healthy control is normal [Figure 2a]. There is an important reduction of the lumen of the proximal and distal convoluted tubes of CCl₄-treated rats [Figure 2b] as compared to the healthy animal. Renal section of rats treated with silymarin (25 mg/kg) or aqueous trunk bark extract of *M. tomentosa* (50 or 25 mg/kg) was similar to that of healthy control [Figures 2c-f].

DISCUSSION

This study was designed to evaluate the effects of aqueous trunk bark extract of *M. tomentosa* on CCl₄-induced liver injury in rats since it has been claimed to be used for the management of liver diseases. Results showed that aqueous extract of *M. tomentosa* protects the liver against CCl₄ damages. Administration of CCl₄ occasioned a significant increase in liver enzyme activities, malondialdehyde levels, and induced an important leukocytes infiltration in liver tissue, which was prevented by treatment with *M. tomentosa* aqueous trunk bark extract.

Inflammation is a natural defense reaction of the body against any aggression, whether exogenous or endogenous. It takes place in the vascularized tissues and its ultimate

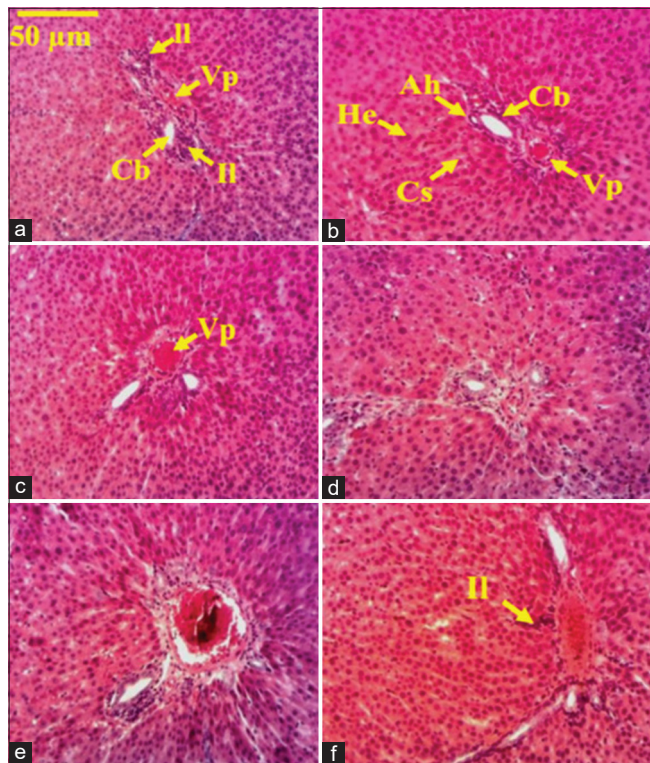


Figure 1: Microphotography of the liver sections of healthy and CCl₄-treated rats under *Markhamia tomentosa* aqueous extract treatment. Histological sections stained with hematoxylin-eosin (×100). (a) Distilled water + CCl₄, (b) distilled water + olive oil, (c) silymarin (25 mg/kg) + CCl₄, (d) ATBE 50 + olive oil, (e) ATBE 50 + CCl₄, and (f) ATBE 25 + CCl₄. VP: Portal hepatic vein, He: Hepatocytes, Cs: Sinusoidal capillary, Ah: Hepatic artery, Cb: Biliary canaliculus, Il: Leukocyte inflammation, NC: Necrosis cell, S: Steatosis. ATBE 50 or 25: Aqueous trunk bark extract of *Markhamia tomentosa* at the dose of 50 or 25 mg/kg, CCl₄: Carbon tetrachloride.

goal is protection against all dangers. The liver, being a highly vascularized organ is, therefore, the privileged site of inflammatory reactions. It is a vital organ that is vulnerable to factors of liver damage, such as toxics and their metabolites, toxins, viruses, high-fat diet, and excessive alcohol consumption.^[27] Liver inflammation caused by CCl₄ is due to the lyse of hepatic cells by free reactive radicals from CCl₄ biotransformation. P450 cytochrome metabolized CCl₄ into CCl₃, which, in turn, is processed in oxygenated condition to the CCl₃OO.^[28] These reactive species may cause harmful effects in the body by oxidizing macromolecules such as DNA, proteins, and polyunsaturated fatty acids. Hence, prolonged exposure to such reactive CCl₄ metabolites leads to oxidative stress which results in the breakdown of membrane structure, disruption of cell energy, and protein synthesis processes, leading undoubtedly to cell injury.

Change in body weight is an adequate index to assess the seriousness of pathologies and to judge the normal

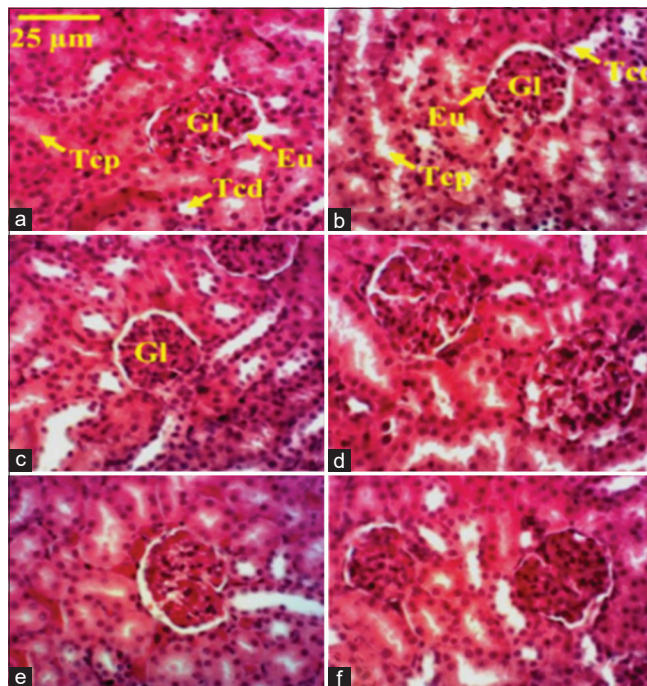


Figure 2: Microphotography of the kidney sections of healthy and CCl₄-treated rats under *Markhamia tomentosa* aqueous extract treatment. Histological sections stained with hematoxylin-eosin (×100). (a) Distilled water + CCl₄, (b) distilled water + olive oil, (c) silymarin (25 mg/kg) + CCl₄, (d) ATBE 50 + olive oil, (e) ATBE 50 + CCl₄, and (f) ATBE 25 + CCl₄. Eu: Urinary space, G: Glomerulus, Tcd: Distal involved tube, Tep: Proximal involved tube. ATBE 50 or 25: Aqueous trunk bark extract of *Markhamia tomentosa* at the dose of 50 or 25 mg/kg, CCl₄: Carbon tetrachloride.

functioning of the body. In addition, the ingestion of toxic substances causes a loss of body weight.^[29] However, the interpretation of the results on the fluctuation of the body mass should not be done in an isolated way but it is necessary to take into account the variation of the mass and the relative mass of the organs. The results obtained show a significant decrease in the body mass of the hepatitis control animals while the mass and the relative mass of their liver do not show any significant difference compared to the healthy group, thus demonstrating liver edema in the hepatitis control. The rats treated with silymarin showed a significant loss of weight at the end of the treatment compared to healthy animals. The average mass of their liver is likewise significantly lower than that of the healthy control, but not their relative mass. By comparing these results with those of the hepatitis controls, it appears that silymarin inhibited edema due to CCl₄. The results show that the hepatitis animals treated with the extract of *M. tomentosa* show a markedly improved growth compared to the hepatitis control rat, thus suggesting a beneficial effect of the extract against CCl₄ hepatitis. At the end of the study, the results indicate that the relative mass of the liver of hepatitis animals treated with the extract is lower

than that of healthy and hepatitis controls. This could suggest an anti-edema effect of the plant extract. However, if we take into consideration the growth of healthy rats treated with the aqueous extract and their low relative liver weight, then we could also suggest that the ability of plant components would cause a reduction of the mass of hepatic tissue; and if so, the mechanisms of this effect remain to be demonstrated.

Hepatitis caused by CCl₄ is characterized by an increase in plasma levels of ALT and AST.^[30,31] Transaminases (ALT and AST) are enzymes found in body cells, particularly in the liver and muscles. An increase of these enzymes in the blood is an indication of hepatic cells lysis.^[30] Our results showed a significantly increased level of these enzymes when compared to the healthy group. A substantial decrease level of these enzymes was observed in hepatitis rats treated with aqueous extract. These results suggest that aqueous extract contains compounds that may protect liver cells against CCl₄ damage. In general, polyphenol and flavonoid compounds could prevent cell lysis due to free radicals.^[32,33] Gamma-glutamyltransferase (γ GT) is a glycoprotein ubiquitous enzyme mostly found in the renal, hepatic, and pancreatic cells. High levels of γ GT in the blood may be a sign of liver disease or damaged bile ducts.^[34] γ GT is more suitable for diagnosing cholangitis, cholecystitis, and obstructive jaundice than alkaline phosphatase, leucine aminopeptidase, and transaminases. Elevated serum value of this enzyme is also observed in hepatitis induced by CCl₄.^[28] Our results show significant blood elevated activity of the γ GT. This finding is consistent with hepatobiliary disease induced by CCl₄. Serum γ GT activity was significantly reduced following treatment of rats with the aqueous trunk bark extract of *M. tomentosa* as well as silymarin thus proving the efficiency of the plant extract against CCl₄-induced liver damage. Bilirubin is a useful indicator to evaluate the excretory function and dysfunction of hepatic bilirubin metabolism in the hepatic cell.^[35,36] Hepatocytes produce bilirubin from hemoglobin and excrete it in bile or urine in a conjugate form with glucuronic acid.^[35,37] Bilirubin similarly leaks into the bloodstream like transaminases when the biliary system is blocked. Administration of CCl₄ causes an inflammatory reaction that alters liver cells, disorganizes the hepatic parenchyma, and increasing the plasma level of bilirubin.^[35,37] Our results show a significant elevation of serum total bilirubin. Treatment of animals with aqueous trunk bark extract of *M. tomentosa* significantly reduced blood bilirubin level. This result is in agreement with that on γ GT activity, suggesting that extract has a protective effect against CCl₄-induced hepatitis. Plant flavonoid compounds are a gifted class of nutraceuticals that have been reported to be able to protect against hepatic damages.^[30,38] These results revealed an improvement of enzymatic and non-enzymatic parameters thus demonstrating the efficacy of the extract of trunk bark extract of *M. tomentosa* to preserve normal functional liver status.

The endogen antioxidant defense system is composed of superoxide dismutase, catalase, and glutathione peroxidase, which are the main enzymes involved in the protection of the body against oxidative stress, by keeping the redox balance between the pro-oxidant and antioxidant mechanisms.^[39] When the antioxidant system failed to keep pro-oxidant processes under control, this results in lipids peroxidation and alteration of DNA and protein structures.^[40] MDA is the end product of lipid peroxidation widely used to quantify oxidative injury.^[41] Lipid peroxidation is one of the major features of the CCl₄-induced hepatitis.^[41] A significant elevation of MDA level in the liver and kidney of hepatitis control rats was recorded in the present study. These results are indicative of the higher level of lipid peroxidation in these tissues, signifying the failure of endogen antioxidant defense mechanisms to prevent the formation of excessive free radicals involved in cell damages. This suggestion is consistent with the results which indicate a significant depletion in the tissues level of reduced glutathione as a consequence of the reduction of the glutathione peroxidase activity, and a significant lowering of the SOD activity as well. Glutathione peroxidase is a cytosolic enzyme that uses reduced glutathione to catalyze the reduction of hydrogen peroxide or peroxide radicals to water and oxygen or alcohols and oxygen, respectively. This enzyme has a high affinity to H₂O₂ compared to catalase.^[42] The peroxidase and reduced glutathione are considered as major defense system when the oxidative stress is not high.^[43] This study revealed that treating rats with an aqueous trunk bark extract of *M. tomentosa* induce a significant reduction in MDA levels, indicating that this extract reduces membrane lipid peroxidation. The results also demonstrate a substantial rise in catalase activity and a decrease in glutathione rate. Hence, it is assumed that the extract's action may depend on the endogenous antioxidant defense system. However, after treating healthy animals with an aqueous extract of *M. tomentosa*, an increased amount of reduced glutathione was seen, as well as an increase in catalase and SOD activity, resulting in a decreased level of MDA in tissues. The findings of this study demonstrated the preventive efficacy of *M. tomentosa* aqueous extract against CCl₄-induced hepatic oxidative stress.

In this study, the histological alterations of liver tissue recorded in rats subjected to CCl₄ are consistent with the findings of earlier studies, which indicate a major lesion of the hepatic parenchyma like hepatocytes necrosis and inflammatory leukocyte cells infiltration in liver tissue as a result of higher oxidative stress.

It has been shown that the inflammatory reaction elicited by CCl₄ in liver tissue is caused by lipid peroxidation, which increases the permeability of cell membranes and hence induces cell death.^[11] Furthermore, oxidative stress causes the release of cathepsins B and then TNF- α by compromising the integrity of the lysosome membrane.^[11] All these processes,

according to Elgawish *et al.*,^[11] result in an upregulation of the pro-apoptotic protein p53, resulting in an inflammatory response in hepatic tissue. Early evidence suggested that cytokines such as TNF- α , IL-1, IL-6, and IL-10 are involved in the activation and enrolment of inflammatory cells in the liver.^[27,44] The histological evaluation of the liver tissue revealed a significant decrease in the infiltration of inflammatory cells. This finding is consistent with the antioxidant properties early observed, like this, reveals the inhibiting effect of *M. tomentosa* on CCl₄-induced hepatitis. Based on the current findings, *M. tomentosa* aqueous extract may act similarly as silymarin either by maintaining optimal redox stability in the tissue through stimulation of antioxidant and non-enzymatic molecules or by decreasing inflammatory responses mainly through mechanisms that need to be investigated.

The results of this study are consistent with those of Temdie *et al.*^[23] which demonstrated the protective activity of the methanol leave extract of *M. tomentosa* on D-galactosamine/lipopolysaccharide and those of Ibrahim *et al.*^[45] which also reported the prophylactic and therapeutic effects of a plant of the family Bignoniaceae against paracetamol-induced liver damage in rat. These pharmacological activities are suggested to be due to bioactive components of *M. tomentosa* trunk bark aqueous extract such as polyphenols, flavonoids, tannins, saponins, and anthocyanins found in the aqueous trunk bark extract. Some of these major classes of bioactive compounds have been identified in the *Markhamia* genus^[18,45] and their strong antioxidant activities have been proved.^[20,32,33]

CONCLUSION

The aqueous trunk bark extract of *M. tomentosa* has hepatoprotective action against CCl₄-induced liver damage. Its antioxidant and anti-inflammatory properties would be responsible for this effect. Indeed, *M. tomentosa* extract preserves the liver's physiological functions and architecture from the inflammatory process caused by CCl₄ administration, by inhibiting lipid peroxidation, improving the endogen antioxidant defense system, and reducing inflammatory cell mobilization in the injured liver tissue. These findings support the use of *M. tomentosa* by communities to treat liver diseases. Nonetheless, further research is needed at this time to use this plant as an alternative treatment for liver diseases.

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Authors' contributions

All authors have participated to the realization of this work either by conceiving and designing the experiments,

by conducting work on the field and data collecting, by contributing to data analysis, interpretation, and the preparation of manuscript. The final version of the article was approved by authors for publication.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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