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Research Article

Hepatoprotective Activity of Markhamia tomentosa (Benth) K. Schum (Bignoniaceae) Aqueous Leaves Extract against CCl4-Induced Subacute Liver Injury in Rat

Romeo Joel Guemmogne Temdie[1]*, Emmanuel Le Fils Doumarsou Boumzina[1], Marc Germain Kuum Minoue[2], Arnaud Doumogne Djasrane[1], Pierre Jidibe[1], Agathe Lambou Fotio[3], Selestin Dongmo Sokeng[1] and Theophile Dimo[4]

[1]*Department of Biological Sciences, University of Ngaoundere, Ngaoundere, Cameroon*

[2]*Department of Psychology, University of Douala, Douala, Cameroon*

[3]*Department of Zoology and Animal Physiology, University of Buea, Buea, Cameroon*

[4]*Department of Animal Biology and physiology, University of Yaounde I, Yaounde, Cameroon*

***Corresponding Author:** Romeo Joel Guemmogne Temdie, Department of Biological Sciences, Faculty of Sciences, University of Ngaoundere, Cameroon.

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Abstract

Markhamia tomentosa (Bignoniaceae) is used against liver diseases and some pharmacological studies have demonstrated its pharmacological properties. However, only a few studies were focussed on its hepatoprotective effects. Therefore, this work was designed to investigate the hepatoprotective effects of the aqueous leaves extract of *Markhamia tomentosa* on CCl_4-induced sub-acute liver injury in rat.

Thirty albino Wistar rats were dispersed equally into 6 groups, including hepatitis control group (H_2O + CCl_4), healthy control group (H_2O), positive control group (Silymarin 25 mg/kg + CCl_4), extract control group (aqueous leaves extract of *Markhamia tomentosa* 25 mg/kg), two test groups (aqueous leaves extract of *Markhamia tomentosa* 25 mg/kg + CCl_4 or 50 mg/kg + CCl_4, respectively). CCl_4 was injected (0.5 mL/kg, i.p.) on the 4^{th} and 11^{th} days whereas others substances were given orally once a day for 14 days. At the end of the experimental period, all rats were anesthetized and then sacrificed to collect blood, liver and kidney. Hepatotoxicity, nephrotoxicity and oxidative stress were evaluated by measuring the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (γ-GT), bilirubin total, total protein, triglycerides, total cholesterol, creatinine, and malondialdehyde (MDA). Liver and kidney histological alterations were evaluated.

The hepatoprotective effect of aqueous leaves extract of *Markhamia tomentosa* (25 and 50 mg/kg bw) was evident by significant reduction in biomarker enzymes of liver integrity (ALT and AST) and in lipoperoxidation biomarker (MDA) and increase in the liver antioxidative defence capacity (super oxide dismutase, reduced glutathione and catalase) in comparison to the results of the hepatitis control group. Aqueous leaves extract of *Markhamia tomentosa* improved histological changes observed in hepatitis control animals.

These results demonstrated a hepatoprotective effect of the aqueous leaves extract of *Markhamia tomentosa* which may be due to its antioxidant and anti-inflammatory properties. These findings thereby supporting the ethnomedicinal uses of this plant to relieve liver related ailments.

Keywords: *Markhamia tomentosa; Hepatoprotective Effect; Liver Injury; Carbon Tetrachloride; Antioxidant Activity*

Abbreviations

M. tomentosa: *Markhamia tomentosa*; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; γ-GT: Gamma-Glutamyl Transferase; MDA: Malondialdehyde; Sily 25: Silymarin; ALE Mt: Aqueous Leaves Extract of *Markhamia tomentosa*; SOD: Superoxide Dismutase

Introduction

Liver is the second largest organ and one of the most important organs in our body. It is a key organ of the digestive system which performs several vital functions [1]. Liver is a principal site of metabolism of substances which would be secreted in blood, stocked in tissues or excreted out of the body [2].

The primary functions of the liver are production and excretion of bilirubin, bile, hormones, cholesterol, and drugs; metabolism of fats, proteins, and carbohydrates; enzyme activation and synthesis of plasma proteins, such as albumin, and clotting factors; storage of glycogen, vitamins, and minerals; blood detoxification and purification. Because of these important activities, the liver is exposed to a certain number of aggressions which inevitably cause more or less severe lesions [1,3]. Biotransformation is a metabolic process that takes place mainly in the liver to facilitate the elimination of both endogenous and exogenous compounds like drugs or lipid soluble substances. It consist of a series of reaction that modify the chemical structures of these compounds. These enzymatic reactions may result in changes in the pharmacological or toxic activity of the substrates [4]. Enzymes found mostly in cells' cytoplasm, mitochondria, and endoplasmic reticulum, have both constitutive and inducible regulations control and are involve in phase I (reduction, oxidation, and/or hydrolysis) and phase II (conjugation reactions) reactions. In general, biotransformation reactions serve to the detoxication of toxic constituents. However, these may also bioactivate some compounds by transforming them from nontoxic to the toxic form. The effect of these toxic metabolites on the body depends on several factors such as the amount of toxic by-product created, the speed with which the metabolite can undergo a detoxification reaction, its accumulation in the cells, the nature and extent of damage produced by the toxic by-product, as well as factors that delay its excretion [5].

Therefore, in performing its functions, the liver is exposed to the toxicity of various substances such as chemicals or their metabolites (CCl_4), prescribed and over-the-counter drugs (paracetamol, antibiotics, psychotropic drugs, lipid-lowering drugs, non-steroidal anti-inflammatory drugs, and chemotherapeutic agents), alcohol and toxins. Infections, autoimmune disorders, and metabolic storage diseases also lead to several acute and chronic liver injuries [2].

Liver diseases are epidemic and endemic diseases that constitute the most important cause of mortality and morbidity in the world [6]. Liver diseases are either of infectious origin (viral, parasitic or bacterial infection) or of toxic origin. Infectious liver diseases are widely studied and easily diagnosed, which enables a more efficient preventive and curative treatment. This is not the case for toxic-related liver diseases. Formal epidemiological data on the incidence and causes of toxic-related liver diseases are rare [7]. Globally, most of the minor liver toxicity cases go unnoticed and even when the cases of liver damage are confirmed, it is not easy to know if, it is of toxic origin or not. The absence of specific techniques for detecting toxic liver injury forces us to adopt an approach based on the history and the diagnosis of elimination or other etiologies, except in situations of exposure to a high dose of a toxic substance whose hepatotoxic effects are known. These factors may also account for the difficulty of providing precise epidemiological data on each form of toxic liver injury [7].

The situation is becoming increasingly alarming, given the exposure of populations to various agents potentially harmful to the liver and moreover if we have to take into account the combination of toxic substances, given the existence of polyintoxication and additive or synergistic hepatotoxic effect of these substances. Hepatotoxic compounds have been shown to cause approximately 10% of acute liver failure and 5% of jaundice [7].

In the United States, the first cause of liver failure leading to liver transplantation is intoxication by hepatotoxic substances [8]. Lee showed that in the United States, drug-induced hepatitis is more common and causes more than 50% of cases of acute liver failure [9]. A study on the incidence of drug-induced hepatitis injuries in a French region found an annual incidence of 14 cases per 100,000 inhabitants, with a female/male ratio of 0.9 before the age of 49 years and 2.6 after 50 years. The hospitalization rate was 12% and the mortality rate 6% [7]. Xiao., *et al*. stated that 4% of worldwide mortality which correspond to approximately 2.5 million deaths is due to alcohol induced hepatitis [10]. Tsague., *et al*. highlighted the fact that non-viral hepatitis with a prevalence of 13% was a serious case of concern in the Menoua Division, western region of Cameroon [11].

Therefore, treatments for liver disease are the main concern of nowadays research. Any potential source of drugs should be thoroughly explored for therapeutic purposes. An attention is drawn to the potentials of medicinal plants that may have the hepatoprotective ability, in order to work towards the development of specific and innovative treatments.

Some raisons prompt us to explore medicinal plant to meet health needs. Firstly, they have been used since ancient times and up to-now for managing diseases. Secondly, they are an inexhaustible source of molecules that have not yet revealed all of their secrets and have also contributed significantly to the development of current drugs [2].

Markhamia tomentosa (Bignoniaceae) is one amount numerous medicinal plants widely used for its healing virtues [12-15]. Alkaloids, saponins, tannins, cardiac glycosides, anthraquinones, phenols, glycosides, and flavonoids were found in *M. tomentosa* methanol leaves extract and 2-acetyl-6-methoxynaphtho[2,3-b] furan-4,9-dion and 2-acetylnaphtho[2,3-b]furan-4,9-dione were found in ethyl-acetate leaf extract [16,17]. Some studies has demonstrated that its pharmacological activities. Chronic anti-inflammatory effects and anti-arthritic effects were disclosed [16-20]. A relatively non-toxic effect of aqueous and methanol leaves extract of *M. tomentosa* was established [15]. It has been showed that *M. tomentosa* methanol leaves extract protects rodents against D-galactosamine/lipopolysaccharide-induced acute hepatitis [21]. But as far as we have investigated, we have not found any work on CCl_4-induiced liver injury. Thus, the search for powerful natural remedy against the various hepatitis led us to the exploration of the benefit effects of the aqueous leaves extract of *Markhamia tomentosa* against CCl_4-induiced liver subacute liver injury in rat.

Material and Methods

Plant material

Markhamia tomentosa leaves were collected in Bayangam (West Region, Cameroon), in August 2019. A plant sample was identified in the National Herbarium of Cameroon in comparison with the sample kept there under the number 1974/SRFK. The harvested leaves were cut into smaller pieces and dried in the shade for a week. Drying sheets were powdered and kept in the sealed bottle for extraction procedure. A quantity of powder (58g) of *Markhamia tomentosa* leaves was infused (60°C) in an adiabatic container with 330 ml of distilled water for 24 hours. After decantation, the mixture was filtered. 2 mL of filtrate were evaporated in a drying oven (40°C) to determine the concentration (10 mg/mL) and yield of the extraction (5.41%). The remaining filtrate was kept in a freezer at -20°C for further exploitation [22].

Chemicals and reagents

Silymarin (silybon-140) and formol were acquired from Micro Labs Limited (Bangalore, India). Carbone tetrachloride was bought from Sigma (St. Louis Missouri, USA). All kits for biochemical analysis were obtained from Chronolab Systems S.L. (Barcelona, Spain).

Animals

The animals required for this study were male rats, aged ten weeks, weighing between 140 and 170g. These rats were obtained from the Animal House of the Department of Biological Sciences, University of Ngaoundere-Cameroon. Animals were raised under standard

laboratory conditions (12:12h light/dark cycle, temperature 25.6 ± 5°C, hygrometry 79 **±** 7%), with free access to standard commercial diet and clean water. This study was conducted under standard guideline approved by the Cameroon National Ethical Committee (Reg. No FWAIRD 0001954).

Experimental design

Thirty rats were divided into 6 groups of five rats each, including four control groups: hepatitis control (H_2O+CCl_4), healthy control (H_2O+olive oil), positive control (Silymarin 25 mg/kg+CCl_4), and extract control (25 mg/kg bw of aqueous leaves extract of *Markhamia tomentosa* + olive oil); and two test groups: test 25 and 50 (25 or 50 mg/kg of aqueous leaves extract of *Markhamia tomentosa* +CCl_4). Extract was administered *per os* in a single daily dose for 14 days and animals were weighed every day. Liver injury was induced by intraperitoneal injection of CCl_4 (0.5 mL/kg) mixed with olive oil (1/1), on the 4th and 11th day [22]. On day 15th, after 12 hours of fasting, animals were anesthetized with ethyl ether and were sacrificed. The blood was collected in the dry tubes for the preparation of the serum. Liver and kidneys were also removed, rinsed in saline solution (0.9% NaCl) and weighed. Liver and kidney samples were taken for the preparation of the homogenates (20%) into phosphate buffer solution (1.15M, pH; 7.4) and other liver and kidney samples were stored in 10% formalin for histological study. Serum and supernatant which were obtained by centrifugation of blood and homogenates at 3000 rpm for 20 minutes, collected was kept at -20°C for biochemical analysis.

Assessment of biochemical parameters

The assessment of biomarkers of hepatic and renal function (alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, total bilirubin, total proteins, total cholesterol, triglycerides, and creatinine) was carried out according to the protocol of the Chronolab Systems S.L. kits (Barcelona, Spain), revised in 2017. Oxidative stress parameters such as malondialdehyde, catalase, reduced glutathione, and superoxide dismutase were evaluated [22].

Histopathological analysis

Liver and kidney samples were removed from 10% formalin and were submitted to a dehydration process with ethylic alcohol. After clarification in xylene, samples were soaked in paraffin (60°C) and then placed oriented in moulds which were filled with paraffin to form blocks and a 5 µm section was made with microtome (Reichert-Jung 2030). These sections were dewaxed and rehydrated before staining with haematoxylin-eosin [23]. The stained tissues were then observed under a microscope (Scientico STM-50) equipped with a Celestron 44421 digital camera.

Statistical analysis of results

The results were expressed as mean ± SEM and others as microphotograph. The one-way ANOVA test was performed followed by Dunnett's test, using Graph Pad. Prism software V5.03. The difference was considered statistically significant compared to hepatitis control or healthy control at the 5% level ($p \leq 0.05$) and highly significant at 1% level ($p \leq 0.01$).

Results

Effects of the aqueous leaves extract of *Markhamia tomentosa* on the body weight gain and on relative liver and kidney weight of rats

Results showed normal growth of healthy control animals with a weight gain of 2.72 ± 1.4% and 15.33 ± 2.10%, respectively on the 7th and 14th day. Hepatitis control rats showed a significant ($p < 0.05$) slowdown in growth of -3.06 ± 1.85% and 1.95 ± 2.97%, respectively on

the 7th and 14th day of the study, compared to healthy control group. Treatment of animals with the aqueous leaves extract of *Markhamia tomentosa* (25 and 50 mg/kg) significantly ($p < 0.05$) improved weight growth by 3.68 ± 1.94% or 3.06 ± 1.37%, respectively, on the 7th day, compared to the hepatitis control (Table 1).

Treatment	Body weight gain (%)		Absolute weight (g)		Relative mass (%)	
	Day 7th	Day 14th	Liver	Kidney	Liver	Kidney
H_2O + CCl_4	-3.06 ± 1.85#	1.95 ± 2.97#	4.69 ± 0.47	0.97 ± 0.04	3.18 ± 0.11	0.66 ± 0.03
H_2O + Olive oil	2.72 ± 1.41*	15.33 ± 2.10*	4.89 ± 0.47	0.96 ± 0.04	3.50 ± 0.06	0.68 ± 0.02
Sily 25 + CCl_4	-2.61 ± 0.38##	-4.33 ± 1.51##	4.05 ± 0.44	0.88 ± 0.05	3.16 ± 0.14	0.69 ± 0.02
ALE Mt 25+ Olive oil	7.92 ± 1.71**	19.95 ± 3.21**	5.34 ± 0.50	1.01 ± 0.04	3.32 ± 0.08	0.62 ± 0.01
ALE Mt 25 + CCl_4	3.68 ± 1.94*	7.10 ± 3.71	4.38 ± 0.46	0.91 ± 0.02	3.25 ± 0.10	0.67 ± 0.03
ALE Mt 50 + CCl_4	3.06 ± 1.37*	5.28 ± 3.49	4.95 ± 0.49	0.97 ± 0.09	3.39 ± 0.10	0.66 ± 0.01

Table 1: *Change in body weight gain and organs relative mass of rats treated with aqueous leaves extract of Markhamia tomentosa. Each value represents the mean ± SEM. n = 5. *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. ALE Mt 50 or 25: Aqueous leaves extract of Markhamia tomentosa at 50 or 25 mg/kg.*

Effects of the aqueous leaves extract of *Markhamia tomentosa* on biomarkers of liver and kidney function

Effect of the extract on enzymatic biomarkers

Induction of hepatitis by CCl_4 resulted in a significant ($p < 0.01$) increase in serum alanine aminotransferase activity (771.82 ± 28.48 U/L) compared to the healthy control group (292.63 ± 13.94 U/L). Treatment with aqueous leaves extract of *Markhamia tomentosa* (25 or 50 mg/kg) significantly ($p < 0.01$) reduced the activity of this enzyme in rats serum (94.53 ± 14.44 and 145.2 ± 31.12 U/L, respectively), compared to hepatitis control group. Treatment with Silymarin (0.5 mg/kg) significantly reduced (297.35 ± 58.61 U/L) ALT activity. A significant ($p < 0.05$) reduction at 185.21 ± 36.37 U/L was also observed in animals treated with *M. tomentosa* extract at a dose of 25 mg/kg (Table 2).

CCl_4-treated group showed a significant ($p < 0.01$) increase in plasma aspartate aminotransferase (AST) activity (463.62 ± 58.89 U/L) compared to the healthy control group (265.31 ± 35.91 U/L). The 14-day treatment with the aqueous leaves extract of *Markhamia tomentosa* (25 or 50 mg/kg) significantly ($p < 0.01$) reduced the activity of AST to 220.15 ± 15.57 and 237.1 ± 10.05 U/L, respectively, compared to hepatitis control. Silymarin (25 mg/kg) diminished ($p < 0.05$) the AST activity to 295.28 ± 20.99 U/L (Table 2).

Administration of CCl_4 resulted in a significant increase ($p < 0.01$) in the activity of gamma-glutamyl transferase (46.24 ± 4.31 U/L), compared to the healthy control (23.08 ± 1.21 U/L). Treatment with the aqueous leaves extract of *Markhamia tomentosa* (25 or 50 mg/kg) or with Silymarin (25 mg/kg) resulted in a non-significant reduction of the gamma-GT activity (34.87 ± 8.42; 37.96 ± 3.65 or 32.30 ± 2.12 U/L, respectively), compared to the hepatitis control group. Extract control group showed a significant reduction of the gamma-GT activity (17.88 ± 2.49 U/L), compared to the hepatitis rat (Table 2).

Treatment	ALT (U/L)	AST (U/L)	γ-GT (U/L)
H_2O + CCl_4	771.82 ± 28.48$^{\#\#}$	463.62 ± 58.89$^{\#\#}$	46.24 ± 4.31$^{\#\#}$
H_2O + Olive oil	292.63 ± 13.94**	265.31 ± 35.91**	23.08 ± 1.21**
Sily 25 + CCl_4	297.35 ± 58.61**	295.28 ± 20.99*	32.30 ± 2.12
ALE Mt 25+ Olive oil	185.21 ± 36.37**	223.18 ± 43.68**	17.88 ± 2.49**
ALE Mt 25 + CCl_4	94.53 ± 14.44$^{**\#}$	220.15 ± 15.57**	34.87 ± 8.42
ALE Mt 50 + CCl_4	145.2 ± 31.12**	237.1 ± 10.05**	37.96 ± 3.65

Table 2: *Variation in the enzymatic activity of serum biomarker of liver and kidney of rats treated with aqueous leaves extract of Markhamia tomentosa.*

Each value represents the mean ± SEM. n = 5. $^{*}P < 0.05$, $^{**}P < 0.01$ 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. ALE Mt 50 or 25: Aqueous leaves extract of Markhamia tomentosa at 50 or 25 mg/kg.

Effect of the extract on non-enzymatic biomarkers

Effects on serum bilirubin and total protein levels

A significant ($p < 0.01$) elevated level of the serum total bilirubin was observed in the hepatitis control group (0.81 ± 0.02 mg/dL), compared to the healthy control (0.36 ± 0.08 mg/dL). Treatment with aqueous leaves extract of *Markhamia tomentosa* (25 or 50 mg/kg) non-significantly reduced serum bilirubin to 0.64 ± 0.08 and 0.66 ± 0.06 mg/dL, respectively, compared to hepatitis control. Treatment with Silymarin (25 mg/kg) significantly reduced bilirubin serum level to 0.45 ± 0.04 mg/dL (Figure 1A).

No significant difference was observed in the hepatitis control group (6.22 ± 0.51 mg/dL) compared to the healthy control (5.35 ± 0.71 mg/dL) at the end of the experimentation. Administration of aqueous leaves extract of *Markhamia tomentosa* (50 mg/kg) resulted in a significant increase ($p < 0.01$) in the serum level of total proteins (9.60 ± 0.57 mg/dL), compared to hepatitis control and healthy control. A significant increase in serum protein level was also observed in the extract control group (8.96 ± 0.42 mg/dL), compared to the healthy control groups (Figure 1B).

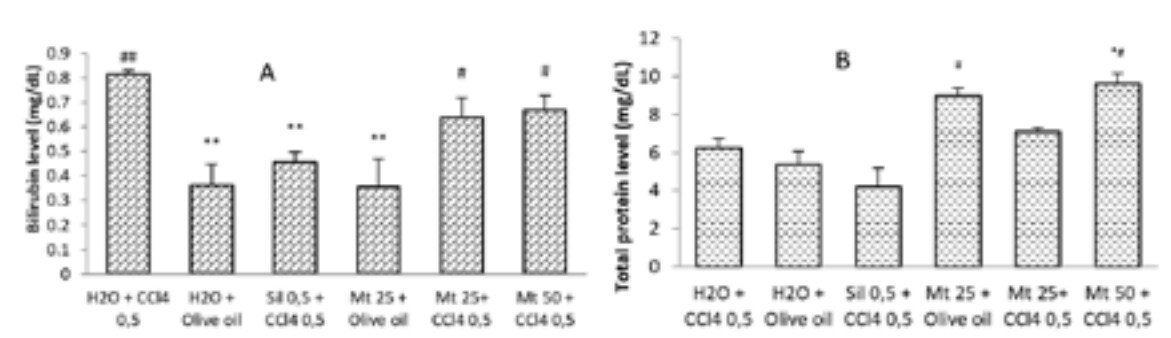

Figure 1: *Influence of the aqueous leaves extract of Markhamia tomentosa on rat's total bilirubin (A) and total protein (B) serum levels. Each bar represents the mean ± SEM. n = 5. *P < 0.05, **P < 0.01 compared to hepatitis control group, #P < 0.05, compared to healthy control group. Sily 25: Silymarin at 25 mg/kg. ALE Mt 50 or 25: Aqueous leaves extract of Markhamia tomentosa at 50 or 25 mg/kg.*

Effect on serum creatinine triglycerides and total cholesterol levels

CCl_4-induced hepatitis initiated non-significant elevation in creatinine level (0.61 ± 0.05 mg/dL) compared healthy control group (0.59 ± 0.05 mg/dL). After treatment with aqueous leaves extract of *Markhamia tomentosa* of (25 mg/kg) the creatinine serum level reduced significantly ($p < 0.05$) (0.44 ± 0.03 mg/dL), compared to the hepatitis control (Figure 2).

Total cholesterol significantly raised in hepatitis rats (83.50 ± 4.73 mg/dL) compared to the healthy control group (58.51 ± 2.67 mg/dL). Treatment with the aqueous leaves extract of *Markhamia tomentosa* (25 or 50 mg/kg) did not cause significant variation (85.17 ± 1.34 or 85.51 ± 7.44 mg/dL) compared to hepatitis control. However, treatment with Silymarin significantly reduced the serum level of total cholesterol to 63.24 ± 5.49 mg/dL (Figure 2). Serum level of triglycerides was not significantly changed following treatment.

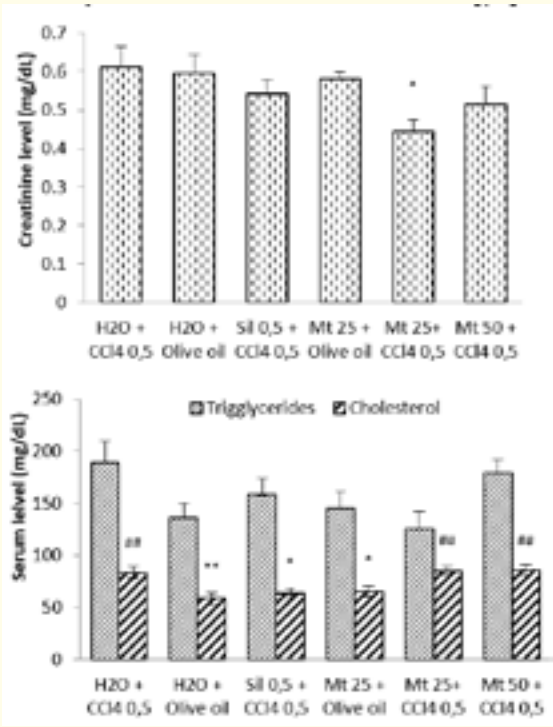

Figure 2: *Change in the creatinine, triglycerides and total cholesterol serum levels of rats treated with aqueous leaves extract of Markhamia tomentosa.*

*Each value represents the mean ± SEM. n = 5. *P < 0.05, **P < 0.01 compared to hepatitis control group, ##P < 0.01 compared to healthy control group. Sily 25: Silymarin at 25 mg/kg. ALE Mt 50 or 25: Aqueous leaves extract of Markhamia tomentosa at 50 or 25 mg/kg.*

Effects of the aqueous leaves extract of *Markhamia tomentosa* on catalase activity, reduced glutathione level, superoxide dismutase activity and malondialdehyde level

Administration of CCl_4 resulted in a non-significant increase of catalase activity in the liver (103.31 ± 4.30 U/mg) and the kidney (75.97 ± 7.77 U/mg), compared to the healthy control (83.19 ± 4.66 or 67.16 ± 7.69 U/mg, respectively). Treatment with the aqueous leaves

extract of *Markhamia tomentosa* at the dose of 25 elicited a significant ($p < 0.05$) increase in liver catalase activity (138.85 ± 13.63 U/mg), compared to the hepatitis and healthy controls. A significant increase ($p < 0.01$) in liver catalase activity (128.50 ± 2.69 U/mg) was observed after administration of silymarin (25 mg/kg). An increase ($p < 0.01$) in catalase activity (137.37 ± 16.33 U/mg) was observed in the kidney following treatment with *M. tomentosa* (50 mg/kg), compared to the hepatitis and healthy controls (Figure 3A).

Administration CCl_4 caused a reduction ($p < 0.01$) of the reduced glutathione level in the liver (264.70 ± 7.12 nmol/mg protein), compared to the healthy control (366.71 ± 4.39 nmol/mg protein). Treatment with the aqueous leaves extract of *Markhamia tomentosa* (25 mg/kg) triggered a significant increase ($p < 0.01$) in the liver level of reduced glutathione (407.94 ± 15.73 nmol/mg protein) as compared to hepatitis rat. Silymarin increased ($p < 0.05$) reduced glutathione level (339.84 ± 6.30 nmol/mg protein) compared to hepatitis rat. In the kidney no significant change in the level of reduced glutathione was noticed (174.11 ± 7.24 nmol/mg protein), compared to the healthy control (222.54 ± 11.81 nmol/mg protein). Administration of the aqueous leaves extract of *Markhamia tomentosa* (50 mg/kg) caused a significant increase ($p < 0.01$) in the liver level of reduced glutathione (285.09 ± 36.06 nmol/mg protein), compared to controls (Figure 3B).

A significant reduction ($p < 0.01$) in the liver activity of superoxydismutase (26.58 ± 0.60 U/mg protein), compared to the healthy control (48.65 ± 0.47 U/mg protein) was observed due to injection of CCl_4. The aqueous leaves extract of *Markhamia tomentosa* at a dose of 50 mg/kg caused a significant increase ($p < 0.01$) in the level of SOD in the liver of rats (42.05 ± 1.60 U/mg protein), compared to the hepatitis control (Figure 3C).

Injection of CCl_4 resulted in a significant ($p < 0.01$) increase in tissue levels of malondialdehyde in liver (3.13 ± 0.02 mmol/mg protein) and kidney (2.75 ± 0.02 mmol/mg protein, respectively), compared to healthy control (2.48 ± 0.01 or 1.23 ± 0.00 mmol/mg protein, respectively). Treatment with aqueous leaves extract of *Markhamia tomentosa* at 25 and 50 mg/kg significantly reduced hepatic (2.58 ± 0.05 or 2.48 ± 0.077 mmol/mg protein, respectively) and renal (1.48 ± 0.02 or 1.49 ± 0.05 mmol/mg protein, respectively) level of MDA, compared to hepatitis control. Silymarin (25 mg/kg) prevented a significant increase in hepatic (2.36 ± 0.06 mmol/mg protein) and renal (1.33 ± 0.00 mmol/mg protein) levels of MDA, compared to the hepatitis control (Figure 3D).

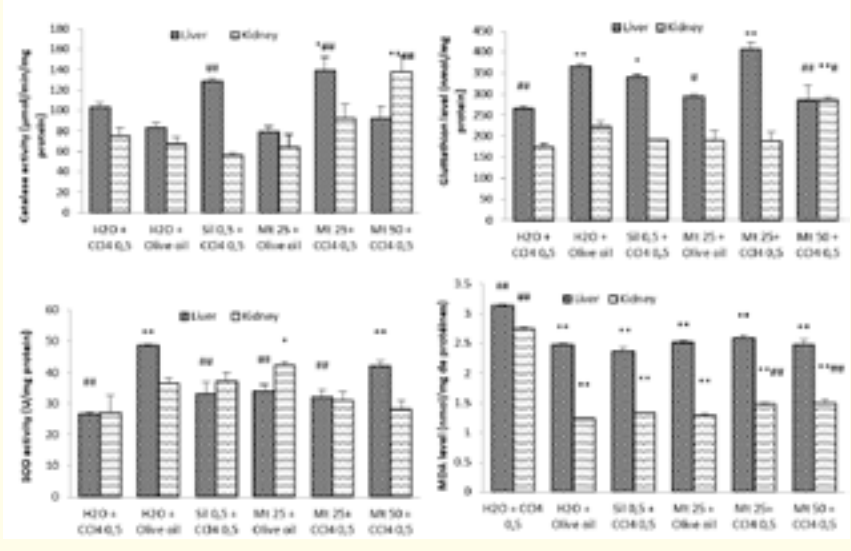

Figure 3: *Change in the catalase activity, reduced glutathione level, superoxide dismutase activity, and malondialdehyde level of rat treated with aqueous leaves extract of Markhamia tomentosa.*

*Each bar represents the mean ± SEM. n = 5. *P < 0.05, **P < 0.01 compared to hepatitis control group, ##P < 0.01 compared to healthy control group. Sily 25: Silymarin at a dose of 25 mg/kg. ALE Mt 50 or 25: Aqueous leaves extract of Markhamia tomentosa at the dose of 50 or 25 mg/kg.*

Effect of the aqueous leaves extract of *Markhamia tomentosa* on CCl_4-induced hepatic lesions

Effect of the extract on hepatic inflammatory lesions

Microphotography of a healthy rat liver section shows normal hepatocytes uniformly coloured and well-organized around the portal space with one or two round nuclei. The various vessels that make up the liver can be clearly seen at the level of the portal space. Sinusoidal capillaries are discernible between rows of hepatocyte cells (Figure 4B). Liver of rats treated with CCl_4 shows a remarkable invasion of the portal space and the entire hepatic parenchyma, by leukocyte cells in the form of a large dot. The hepatocyte parenchyma shows dilated hepatic portal vein filled with blood where many leukocytes can be seen. Necrosis of hepatocytes is characterized by the presence of cell nuclei completely devoid of chromatin, dark in appearance compared to healthy cells and without clearly visible cytoplasmic delimitation (Figure 4A). Treatment of animals with silymarin (25 mg/kg) or the aqueous extract (25 or 50 mg/kg) resulted in a reduction in necrosis and leukocyte infiltration compared to hepatitis controls, thus reflecting a clear improvement in histological parameters (Figure 4C-4F).

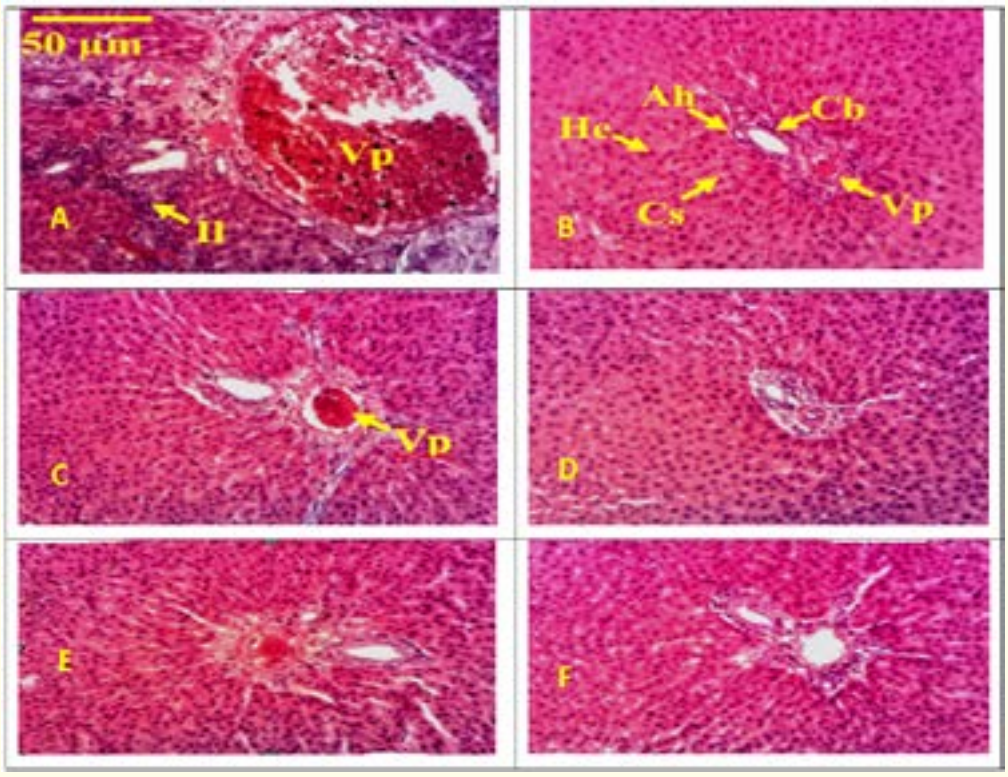

Figure 4: *Microphotography of the liver of rats after treatment with aqueous leaves extract of Markhamia tomentosa. Histological sections stained with haematoxylin eosin (X100), A: Hepatitis control (H2O + CCl4), B: Healthy control (H2O + Olive oil), C: Positive control (Sily 25 + CCl4), D: Extract control (ALE Mt 25+ Olive oil), E: Test 50 (ALE Mt 50 + CCl4) and F: Test 25 (ALE Mt 25 + CCl4). Sily= Silymarine; Vp= Hepatic portal vein; He= Hepatocyte; Cs= Sinusoid capillary; Ah= Hepatic artery; Cb= Biliary duct; Il= Leukocyte infiltration.*

Effect of the extract on renal inflammatory lesions

Observation of the histological section of the kidney suggests a slight alteration which results from a clearly appreciable reduction in the lumen of the proximal and distal convoluted tubes in the hepatitis rat (Figure 5A), compared to the healthy control (Figure 5B). Treatment of rats with the aqueous leaves extract of *Markhamia tomentosa* and silymarin corrected these abnormalities (Figure 5C-5F).

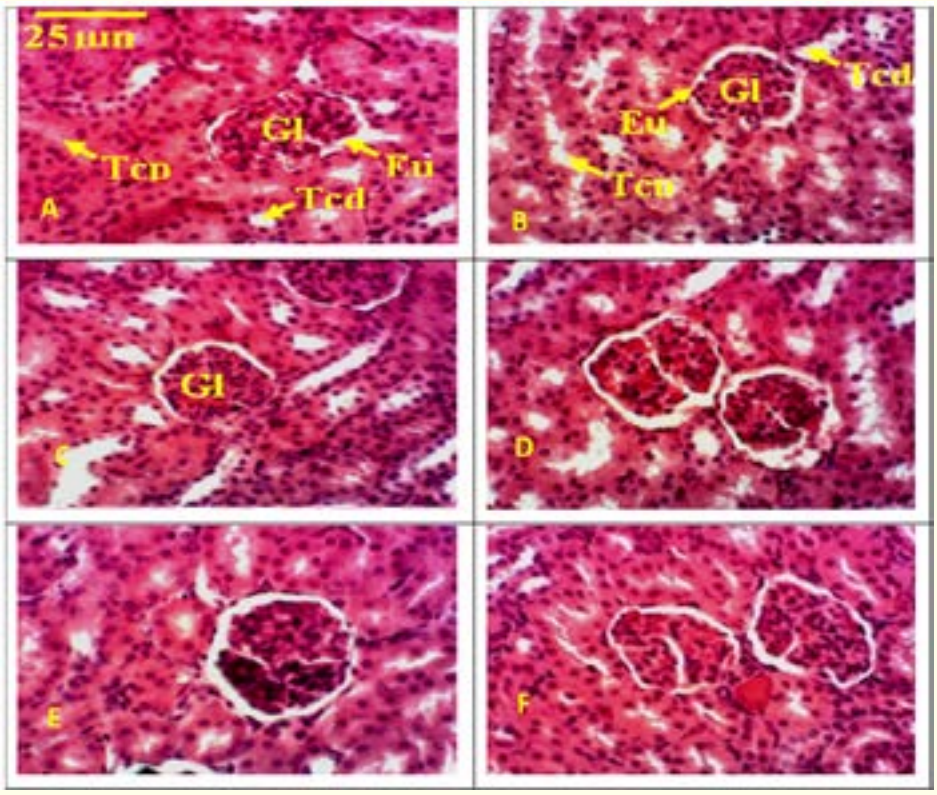

Figure 5: *Microphotograph of the kidney of rats after treatment with aqueous leaves extract of Markhamia tomentosa. Histological sections stained with haematoxylin eosin (X200), A: Hepatitis control (H2O + CCl4), B: Healthy control (H2O + Olive oil), C: Positive control (Sily 25 + CCl4), D: Extract control (ALE Mt 25+ Olive oil), E: Test 50 (ALE Mt 50 + CCl4) and F: Test 25 (ALE Mt 25 + CCl4). Sily= Silymarin, Gl= Glomerulus; Eu= Urinary tract; Tcd= Distal contoured tubule; Tcp= Proximal contoured tubule.*

Discussion

Based on claims of traditional healers and some pharmacological studies done on different extracts of *Markhamia tomentosa*, this study intended to evaluate the effects of aqueous leaves extract of *Markhamia tomentosa* of on liver injury induced by CCl_4 in rats. Results showed that this aqueous extract protects rats against CCl_4-induced liver injury. Aqueous leaves extract of *Markhamia tomentosa* reduced ALT, AST and gamma-GT serum activity, MDA level as well as leucocyte infiltration in liver tissue and increased catalase and GSH concentration in CCl_4-treated rat liver. These study showed that the extract prevented hepatocellular lysis, stimulated endogenous antioxidant system and reduced inflammation activities due to carbon tetrachloride.

The liver is a vitally important organ for our body. It is a richly vascularized tissue that performs an important metabolite function making the liver the centre of biotransformation reactions for the conversion of endogenous or exogenous products into metabolites useful for the proper functioning of the body or into waste compounds to be eliminated by the liver or other organ specialized in excretion [2].

Liver plays principal role in detoxification of various drugs and xenobiotics. Indeed, all exogenous fat-soluble substances are metabolized via reactions of phase I, and phase II [2]. Accomplishment of the detoxification function would expose the liver to lesions. The nature and severity of these lesions could depend on the toxicity of the metabolites obtained. This situation inevitably leads either to acute or chronic hepatitis. The liver has a great capacity for regeneration against many lesions caused by different pathologies. However, permanent exposure to infections, alcohol, autoimmune, various toxins (microcystin), chemicals (carbon tetrachloride, thioacetamide, D-galactosamine, Acryl amide, pyrrolizidine alkaloid, mercury and lead, antitubercular agent like rifampicin, isoniazid and pyrazinamide), and prescribed drugs (tamoxifen, antibiotics such as erythromycin, chemotherapeutic agents like adriamycin) or cum over-the-counter drugs (paracetamol) could ultimately lead to several liver insults like severe hepatitis accompanied by fibrosis, cirrhosis, tumor and subsequent hepatocarcinomas, with loss of the liver function [2].

CCl_4 is an industrial chemical used for many purposes (dry cleaning or fire extinguisher fluid, grain fumigant, and for degreasing metals), causes both acute and chronic liver hepatitis after getting metabolized in the liver. CCl_4 is metabolized by specific ferrous cytochrome P-450 into $CCl_3^{\cdot}$ (trichloromethyl radical) which is then oxidized into $CCl_3O_2^{\cdot}$ (trichloromethylperoxy radical), a highly reactive oxidative free radical that induces peroxidation of polyunsaturated fatty acids of biological membranes or covalently binds to macromolecules in the cell. These process lead to inhibition of proteins synthesis and increase in membrane permeability resulting in hepatocyte necrosis [2]. Our results showed a significant decrease in the body mass of the hepatitis animals compared to healthy group. Change in body weight is an adequate index to evaluate the seriousness of pathologies and to appreciate the normal functioning of the body. Thus, a loss of body mass is indicative of a state of dysfunction within an organism [15]. Animals treated with the *M. tomentosa* leaves extract showed a markedly improved growth compared to hepatitis rat, suggesting beneficial effect of the extract against CCl_4-induced hepatitis.

Certain blood parameters such as enzymes (alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transferase), bilirubin and creatinine can be measured in order to detect liver and kidney damage. These enzymes have a metabolic activity inside the cells. The increase in their plasma level indicates cell damage in the liver, and sometimes in the muscles or kidneys [24]. Hepatitis instigated by CCl_4 injection to rat is characterized by an increase in plasma levels of ALT, AST and gamma-GT [25,26]. Our results displayed a significantly increased level of these enzymes in hepatitis group when compared to the healthy group. Administration of aqueous leaves extract of *Markhamia tomentosa* decrease considerably the level of ALT and AST compared to hepatitis control rats.

We noticed that the levels of ALT and AST are slightly lower than those of normal controls, these variations could be explained either by the amplification of the elimination processes of such enzymes or by other mechanisms that we will try to elucidate in the next studies. Gamma GT level is high when bile ducts is damaged and therefore is considered as a more suitable means for diagnosing cholangitis, cholecystitis, and obstructive jaundice than transaminases, leucine aminopeptidase, and alkaline phosphatase [27]. Elevated activity of gamma GT recorded in this study suggest that the hepatic lesions has led to hepato-biliary obstruction. Treatment with the aqueous leaves extract of *Markhamia tomentosa* or silymarin prevented significant raising in serum gamma GT activity. Thus, proving the effectiveness of the plant extract against CCl_4-induced subacute hepatitis. However, the lack of specificity of the elevation of gamma GT activity justifies a very rigorous approach in order to know the etiology of the increase in gamma GT activity. Our results showed a significant elevation of serum total bilirubin compared to healthy control. Treatment of animals with aqueous leaves extract of *Markhamia tomentosa* did not significantly reduced blood bilirubin level compared to all controls. The results showed a significant reduction in creatinine levels after treatment of the animals with the aqueous leaves extract of *Markhamia tomentosa* compared to hepatitis control. Bilirubin is produced in the spleen and bone marrow and transported to the liver for conjugation to gluconic acid before elimination. The elimination of conjugated

bilirubin by the feces is the most important in the healthy animal compared to the excretion in the urine [28,29]. An inflammatory reaction due to CCl_4, alters liver hepatocytes, disorganizes the hepatic parenchyma and leading to the increasing of total bilirubin plasma level [30]. These observations would allow us to suggest that bilirubin excretion is disturbed, may be by alteration of the hepatic biliary flow or the bilirubin conjugation. Temdie., *et al*. demonstrated that methanol extract of *M. tomentosa* leaves significantly reduced bilirubin levels in mice treated with D-galactosamine/Lipopolysaccharides [21]. This discrepancy in results would be explained by the hepatitis model and dose levels used in these studies. It is important to determine the fraction of unconjugated bilirubin (related to excess production, or to a conjugation deficiency in the liver cell), and that of conjugated bilirubin (due to cholestasis) to discuss these results.

The liver is also the site of protein synthesis. An adequate serum protein level indicates normal physiological activity of the liver and kidney [31]. The determination of total plasma protein is used to monitor metabolic and nutritional diseases, to assess the dysfunction of the immune system and the degree of hydration of the body [32]. Thus, an abnormally high level of blood protein would indicate dehydration or an increase in the gamma globulin (antibody) fraction due to inflammatory diseases. Albumin and immunoglobulins are the most abundant plasma proteins. The albumin level decreases during chronic liver disease [31]. However, increased immunoglobulins are common in chronic liver disease and can cause an increase in total plasma protein concentration, even if albumin is decreased [33]. Administration of aqueous leaves extract of *Markhamia tomentosa* resulted in a significant increase in the serum level of total proteins, compared to healthy control. These results showed that extract would stimulate the production of total proteins and the mechanisms of such activity remain to be investigated. Previous work has shown an increase in plasma proteins after treatment of mice with methanol extract of *M. tomentosa* leaves compared to hepatitis group [21].

In the body, the antioxidant system opposes the peroxidation of polyunsaturated lipids by free radicals and prevents the accumulation of malondialdehyde [34]. In this study, CCl_4 intoxication of animals resulted in a significant increase in tissue MDA levels and a significant reduction in SOD activity and reduced glutathione levels compared to the healthy control. Treatment with the plant extract (25 and 50 mg/kg) stimulated a significant increase in the tissue activity of SOD, catalase, and reduced glutathione level which resulted in a significant reduction of the tissue level of MDA. These results can be explained by mechanisms that contribute to the protection of hepatocytes against free radicals, either by the scavenging of free radicals by the compounds contained in the extract, or by the stimulation of the endogenous antioxidant defence system.

Free radicals, resulting from the hepatic metabolism of CCl_4, create tissue lesions. These lesions will lead to the release of pro-inflammatory mediators such as histamines, interleukins and tumor necrosis factor. These mediators will cause a local vasodilatation, an increase in capillary permeability and leukocyte infiltration of the liver tissue [23]. The histological results showed that carbon tetrachloride intoxication caused leukocyte infiltration of the hepatic parenchyma and hepatocyte necrosis, which corroborates the biochemical results. Treatment with the aqueous leaves extract of *Markhamia tomentosa* (25 and 50 mg/kg) reduced leukocyte infiltration compared to the hepatitis control, suggesting that the extract would have anti-inflammatory effects. These results corroborate the work of Temdie., *et al*. who showed that the extract of *Markhamia tomentosa* leaves has anti-inflammatory activity [17]. According to Yoon and Baek, these activities would be due to the presence of phenolic compounds, especially flavonoids, which are real scavengers of free radicals [35]. Phytochemical investigations have shown that *Markhamia tomentosa* leaves contain alkaloids, flavonoid quinones, saponins, tannins, steroids, coumarin and phenols [16,17,36]. Some flavonoids reduce lipid peroxidation by scavenging free radicals, increasing cellular glutathione content, and regulating nuclear gene expression and are also able to inhibit the transformation of stellate hepatocytes into myofibroblasts, a process responsible for the deposition of collagen fibres leading to cirrhosis [37].

Conclusion

This research shows that the aqueous leaves extract of *Markhamia tomentosa* inhibits CCl_4-induced liver damage in rats by improving weight growth, reducing serum transaminase activity, and gamma-glutamyl transferase. These results highlight the hepatoprotective

effects of the aqueous leaves extract of *Markhamia tomentosa* which would be due to its antioxidant and anti-inflammatory properties. Indeed, the aqueous leaves extract of *Markhamia tomentosa* enhanced the level of reduced glutathione, the activities of catalase and SOD, inhibited lipid peroxidation, and prevented hepatocellular necrosis and the accumulation of inflammatory cells in the hepatic parenchyma. These results support the ethnopharmacological uses of *Markhamia tomentosa* against liver diseases. However, further research is needed to identify the active compounds of this extract and to propose an alternative treatment for liver diseases.

Declarations of Interest

The authors proclaim that there is no conflict of interest regarding the publication of this article.

Authors' Contributions

Temdie Guemmogne Romeo Joel, Sokeng Dongmo Selestin and Dimo Theophile conceived and designed experiments. Temdie Guemmogne Romeo Joel, Boumzina Doumarsou Emmanuel Fils, Djasrane Doumogne Arnaud, and Jidibe Pierre conducted the experiments and collected all data. Temdie Guemmogne Romeo Joel, Boumzina Doumarsou Emmanuel Fils, Minoue Kuum Marc Germain and Fotio Lambou Agathe contributed to data analysis, interpretation and manuscript preparation. All authors have read and agreed final version of the article and gave their approval for publication.

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Ethical Approval

All authors hereby declare that experiments were conducted in agreement with the revised principles of laboratory animal care (NIH publication No. 85-23, 1985) as well as in accordance with an institutional protocol approved by the Cameroon National Ethical Committee (Reg. No FWAIRD 0001954).

Bibliography

1. Benhamou J and Erlanger S. "Maladie du foie et des voies biliaires". 5th edition. Paris: Flammarion Medicine Science (2008).
2. Ansari S., *et al*. "Experimental models and hepatotoxic agents used to study hepatoprotective effect of traditional drugs". *International Journal of Advances in Pharmacy Medicine and Bioallied Sciences* 2.3 (2014): 67-74.
3. Maximas H., *et al*. "A Review on Hepatoprotective Activity of Commonly Consumed Vegetables". *Der Pharmacia Lettre* 5.5 (2013): 290-304.
4. Almazroo OA., *et al*. "Drug Metabolism in the Liver". *Clinical Liver Disease* 21.1 (2017): 1-20.
5. Phang-Lyn S and Llerena VA. "Biochemistry, Biotransformation". StatPearls Publishing LLC (2021).
6. Irfan MA and Kumar M. "A Recent Update on Hepatoprotective Potential of Herbal Plant". Suresh Gyan Vihar University". *International Journal of Environment, Science and Technology* 1.1 (2015): 25-50.
7. Mégarbane B., *et al*. "Foie toxique : mécanismes lésionnels et thérapeutiques pharmacologiques spécifiques". *Réeanimation* 16 (2007): 632-642.

8. Navarro VJ and Senior JR. "Drug-related hepatotoxicity". *New England Journal of Medicine* 354 (2006): 731-739.

9. Lee W. "Drug-Induced Hepatotoxicity". *New England Journal of Medicine* 349.5 (2003): 474-485.

10. Xiao J., *et al*. "A novel antioxidant multi target Iron Chelator M30 Protects Hepatocytes against Ethanol-Induced Injury". *Hindawi Published Corporation* 60727 (2015): 1-11.

11. Tsague MK., *et al*. "Prevalence of viral and non-viral hepatitis in Menoua Division, West Region, Cameroon: a retrospective hospital-based study". *Pan African Medical Journal* 32 (2019): 212.

12. Bouquet A and Debray M. "Plantes medicinales de la Cote d'Ivoire". ORSTOM, Paris, (1974).

13. Burkill HM. "The useful plants of West Tropical Africa". 2nd edition. Royal Botanic Gardens, Kew, UK. (1985).

14. Arbonnier M. "Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest". 2nd edition, CIRAD, MNHN, Montpellier (2000).

15. Temdie RJG., *et al*. "Safety assessment of *Markhamia tomentosa* (Benth.) K. Schum. (Bignoniaceae) leaves extracts, highlight the psychostimulant effect of the methanol extract". *Journal of Experimental and Applied Tropical Biology* 1 (2021): 37-47.

16. Tantangmo F., *et al*. "Antiprotozoal activities of some constituents of *Markhamia tomentosa* (Bignoniaceae)". *Annals of Tropical Medicine and Parasitology* 104 (2010): 391-398.

17. Temdie RJG., *et al*. "Analgesic and anti-inflammatory effects of the leaves of *Markhamia tomentosa* (Benth.) K.Schum. (Bignoniaceae)". *Pharmacologia.* 3 (2012): 565-573.

18. Aladesanmi AJ., *et al*. "Antimicrobial and antioxidant activities of some Nigerian medicinal plants". *African Journal of Complementary and Alternative Medicines* 4 (2007): 173-184.

19. Adebayo AH., *et al*. "Hepatoprotective activity of chrysophyllum albidum against Carbon tetrachloride induced hepatic damage in rats". *Canadian Journal of Pure and Applied Sciences* 3 (2011): 1597-1602.

20. Temdie RGJ., *et al*. "The effects of the methanol leaf extract of *Markhamia tomentosa* (benth.) k. schum. (bignoniaceae) on arthritis induced by complete freund's adjuvant in rats". *World Journal of Pharmacy and Pharmaceutical Sciences* 5 (2016): 79-92.

21. Temdie RJG., *et al*. "Protective Activity of *Markhamia tomentosa* (Benth.) K. Schum. (Bignoniaceae) Methanol Leaves Extract against D-galactosamine/Lipopolysaccharide-induced acute liver injury in mice". *Journal of Biological Sciences and Medicine* 8 (2020): 74-89.

22. Temdie GRJ., *et al*. "Influence of aqueous leafy stem extract of *Cochlospermum tinctorium* A. Rich. (Cochlospermaceae) on liver injury induced by subacute exposure of rats to carbon tetrachloride". *American Journal of Biopharmacy and Pharmaceutical Sciences* 2 (2022): 1-14.

23. Fotio LA., *et al*. *"Bidens pilosa* Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice". *EC Pharmacology and Toxicology* 7.11 (2019): 119-131.

24. Bruno R. "Les Dosages sanguins liés aux maladies hépatiques". Centre Hépato-biliaire Paul Brousse". *Revue Francophone Des Laboratoire* 48 (2016): 127-134.

25. Ragab A., *et al*. "Protective effect of glycyrrhizc acid against carbon tetrachloride-induced liver fibrosis in rats: integrin subunit β like 1 (itg β l1)". *Slovenian Veterinary Research* 56.22 (2019): 673-679.

26. Kim TW., *et al*. "Hepatoprotective effects of polymethoxyflavones against acute and chronic carbon tetrachloride intoxication". *Food and Chemical Toxicology* 91 (2016): 91-99.

27. Payen JL and Robic MA. "Conduite à tenir devant une élévation des gamma-glutamyltransférases". (2007).

28. Al-Harbi N., *et al*. "Carbon tetrachloride-induced hepatotoxicity in rat is reversed by treatment with riboflavin". *Intenatonal journal of Immunopharmacolgy* 21 (2014): 383-388.

29. Ajith A., *et al*. "Zingiber officinale Roscoe prevents acetaminophen induced acute toxicity by enhancing hepatic antioxidant status". *Food Chemistry and Toxicology* 45 (2011): 2267-2272.

30. Sasidharan S., *et al*. "In vitro antioxidant activity and hepatoprotective effects of *Lentinula edodes* against paracetamol induced hepatotoxicity". *Molecules* 15 (2010): 4478-4489.

31. Marshall WJ and Bangert SK. "Clinical Chemistry". 5th Edition. Elsevier, London, UK (2005).

32. Estepa L. "Protéines totals". Elsevier Masson SAS (2006).

33. Eckersall, PD. "Proteins, proteomics and the dysproteinemias". In Kaneko, J.J., Harvey, J.W., Bruss M.L., Clinical biochemistry of domestics animals, 6th edition, San Diego (2008).

34. Singh D., *et al*. "Modulatory potential of α-amyrin against hepatic oxidative stress through antioxidant status in wistar albino rats". *Ethnopharmacology* 161 (2015): 186-193.

35. Yoon JH and Baek SJ. "Molecular targets of dietary polyphenols with anti- inflammatory properties". *Yonsei Medical Journal* 46 (2005): 585.

36. Fu GM., *et al*. "Naturally occurring phenylethanoid glycosides: Potential leads for new therapeutics". *Curry Medecine Chemmestry* 15 (2008): 259-266.

37. Fraschini F., *et al*. "Pharmacology of silymarin". *Clinical Drug Investigation* 22 (2002): 51-65.

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