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Experimental and Mathematical Model for the Hepatoprotective Effect of Methanolic Extract of *Moringa oleifera* Leaf against CCl_4- induced Hepatotoxicity in Sprague Dawley Male Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author MJO designed the study, wrote the protocol and checked the manuscript. Authors AMO and AOR managed literature searches, designed the model and carryout analyses of the data. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: To evaluate the in-vivo antioxidant potential of methanolic extract of *Moringa oleifera* leaf against CCl_{4-} induced toxicity in rats.
Methods: The phytochemicals present in the plant were determined using standard methods. Male albino rats were made hepatotoxic by orally administered with CCl_4 (20% CCl_4 in olive oil) twice per week for a period of four weeks. They were orally treated with *Moringa oleifera* leaf extract (250 and 500 mg/kg body weight) and silymarin (200 mg/kg body weight) once a day for 28 days. Biochemical assays such as: plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total

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cholesterol (TC), triglyceride (TG), total protein (TP), lipid peroxidation, catalase, reduced glutathione, superoxide dismutase, glutathione peroxidise and histopathology were used to assess damage caused by CCl_4 and the protective effects of the extract on the liver tissues. The mathematical model was analysed using Maple 18.0 software

Results: Phytochemical screening of *Moringa oleifera* shows the presence of secondary metabolites like tannin, anthocyanine, steroid, anthraquinones, terpenoids and saponin. The extract does not have any effect on the hematological parameters. The results showed that oral administration of *M. oleifera* significantly reduced (P<0.05) the elevated plasma levels of AST, ALT, ALP, GGT, TC, TG and the level of malondialdehyde in the rats liver that were induced with CCl_4. Treatment with the extract was also found to significantly increase (P<0.05) the TP level, the activities of SOD % inhibition, SOD unit, CAT and GPx, as well as increase the GSH content in the liver of the animals. Liver histopathology also showed that the extract reduced the incidence of liver lesions induced by CCl_4. Mathematical model analysis shows that treating the rats with *Moringa oleifera* leaf extract gives an optimal result just like the standard drug which is in conformity with the experimental result of the bench work.

Conclusion: The result suggested that *M. oleifera* exhibits potent hepatoprotective effects on CCl_4-induced liver damage in rats due to the increase of antioxidant-defence system activity and the inhibition of lipid peroxidation.

Keywords: Biochemical parameters; hepato-protective effects; mathematical model; Moringa oleifera; carbon tetrachloride.

1. INTRODUCTION

Liver is an organ in the body that plays a major role in the regulation of many of the physiological processes like metabolism, secretory and storing functions. It is involved in detoxification of a variety of drugs and xenobiotics [1]. Carbon tetrachloride (CCl4) is widely used as experimental model for liver damage. The hepatotoxicity of CCl_4 is due to reductive dehalogenation products, such as trichloromethyl ($CCl_3\cdot$) and trichloromethyl peroxyl ($CCl_3O_2\cdot$) radicals [2]. These radicals can bind to proteins and lipids or remove a hydrogen atom from unsaturated fatty acids, thereby initiating lipid peroxidation and contributing to liver injury [3]. Hepatocyte injury initiates the activation of Kupffer cells which secrete potent mediators of the early inflammatory response, such as reactive oxygen species (ROS), especially superoxide anions that accounted for the formation of peroxynitrites and hydrogen peroxides (H_2O_2) which lead to oxidative stress [4].

Plants are well known as a major source of traditional and modern medicines. *Moringa oleifera* belongs to the species of *Moringaceae* family. Its leaves are rich in macro- and micronutrients including polyphenols, phenolic acids, vitamins, carotenoids, flavonoids, and alkaloids [5]. *Moringa oleifera* is referred to as “Miracle tree” in tropics and subtropics with a wide range of health benefit. The *leaves* can be cooked, eaten fresh or stored as dried powder for many months without refrigeration and loss of nutritional values [6]. The plant is reported to possess antitumor, antipyretic, anticonvulsant and anti-inflammatory [7]. Various parts of this plant such as the leaves, flower, seed, fruit, roots, bark and immature pods acts as cardiac and circulatory stimulants, possess antiulcer, antispasmodic, antioxidant, antifungal, antibacterial, anti-diabetic, diuretic, antihypertensive, cholesterol lowering capacity and hepatoprotective properties [8-10].

This study investigates the ameliorating potentials of the methanolic leaf extract of *Moringa oleifera* on CCl_4 induced liver damage in Sprague Dawley male albino rats.

2. METHODOLOGY

2.1 Collection and Identification of Plant Material

The leaves of *Moringa oleifera* were obtained from Ikorodu in Lagos State, Nigeria. The plant was authenticated by a botanist from the department of Botany, University of Lagos, Lagos-Nigeria. Authentication number for the *Moringa oleifera* leaf was 6965.

2.2 Preparation of Methanolic Leaf Extract of *Moringa oleifera*

The leaves of *Moringa oleifera* were washed, air dried under shade in the Biochemistry Laboratory,

pulverised to coarse power using blender. Extraction was carried out by dispersing 200 g of the grounded *Moringa oleifera* plant material in 1L of 80% methanol and shaking was done with GFL shaker for 72 hours. This was followed by vacuum filtration and concentrated by rotary evaporator at a temperature not exceeding 40ºC. The concentrated extract was dried to complete dryness in an aerated oven at 40ºC for 48 hours. The extract was later stored in a refrigerator at 4ºC.

2.3 Phytochemical Analysis of Methanolic Leaf Extract of *Moringa oleifera*

Phytochemical tests for bioactive constituents were carried out on portions of the residual material using standard phytochemical procedures [11-13].

2.4 Experimental Animals

A total of 30 male Sprague Dawley albino rats with body weight ranging from 140 to 180 g were obtained from Ratzmattazz Nigeria enterprises, 21 insurance estate satellite Town Lagos, Nigeria. They were acclimatized for two week to Laboratory condition of 23 ±2ºC. They were kept in plastic cages and fed with commercial rat chow and supply with water *adlibitum.* The rats were used in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication revised (1985) NIPRD Standard Operation Procedures (SOPs).

2.4.1 Acute toxicity test

A total number of 15 Sprague Dawley albino rats were used for the test and they were grouped into three groups, after which they were acclimatized and administered the leave extract of *Moringa oleifera* dosage of 10,100, 1000 mg/kg body weight for one day using modified Lorkes method [14].

2.4.2 Grouping of animals

The animals were grouped into six groups. Each group contains five animals. The animals were grouped as follows:

Group A: Normal control
Group B: Negative control (CCl_4 without treatment)
Group C: Positive control (CCl_4 + 200 mg/Kg B.WT silymarin)
Group D: Olive oil only
Group E: CCl_4 + 250 mg/Kg B.WT of *Moringa oleifera* leaf extract
Group F: CCl_4 + 500 mg/Kg B.WT of *Moringa oleifera* leaf extract

2.4.3 Administration of CCl_4

Male albino rats (Sprague Dawley) of about eleven weeks old with weight range of 140-180g were made hepatotoxic by orally administered with CCl_4 (20% CCl_4 in olive oil) dosage of 1mL per kg body weight twice per week for a period of four weeks.

2.5 Collection of Blood Samples

The albino rats were sacrificed by cervical decapitation after 24 hours of fasting. Blood were collected from the male albino rats by ocular puncture into EDTA tubes for hematological analysis and the remaining blood were collected in an heparinised tubes and centrifuge at 3000 rpm for 20 minutes using a centrifuge and the plasma stored at -20ºC.

2.6 Determination of Hematological Parameters

The hemoglobin concentration (HGB), total red blood cell (RBC), white blood cell count (WBC), Hematocrit (HCT), and other hematological parameters were determined in the whole blood using BC-3200 Auto Hematology Analyzer in University of Lagos Teaching Hospitals (LUTH) in Idi-araba, Lagos, Nigeria.

2.7 Measurement of Plasma Liver Biomarker Enzymes and Lipid Profile

Liver damage was assessed by the estimation of plasma activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) using commercially available test kits from Randox Laboratories Ltd. (UK). The results were expressed as units/liter (IU/L). In addition, the plasma levels of total protein (TP), cholesterol (CH) and triglyceride (TG) were estimated in the experimental animals using kits produced by Randox Laboratories Ltd. (UK).

2.8 Hepatic Antioxidant Activities

2.8.1 Preparation of liver homogenate

The Liver tissues of some of the sacrificed albino rats were excised and the liver samples were cut into small pieces and homogenized in phosphate buffer saline (PBS) to give a 10% (w/v) liver

homogenate. The homogenates were then centrifuged at 12,000 rpm for 50 minutes. The supernatant obtained was later used for assay of thiobarbituric acid reactive substances (TBARS) content, superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione.

2.8.2 Estimation of lipid peroxidative (LPO) indices

Lipid peroxidation as evidenced by the formation of TBARS was measured in the homogenate by the method of Niechaus and Sameulsson [15].

2.8.3 Estimation of superoxide dismutase (SOD)

The liver homogenate was assayed for the presence of SOD by utilizing the technique described by Mccord and Fridovich [16].

2.8.4 Estimation of catalase (CAT)

The liver homogenate was assayed for catalase colorimetrically at 620 nm and expressed as µmoles of H_2O_2 consumed/min/mg protein as described by sinha [17].

2.8.5 Estimation of reduced glutathione (GSH)

Reduced glutathione (GSH) was determined in the liver homogenate using the method of Ellma [18].

2.8.6 Estimation of Glutathione peroxidase

Glutathione peroxidase was determined in the liver homogenate using the method of Paglia [19].

2.9 Histopathological Studies

The histopathological analyses were assayed in the Department of Anatomy, college of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria. The albino rats were sacrificed and their abdomens were cut open to remove the liver. Some of the livers were fixed in Boucin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12 hours, and then embedded in paraffin using conventional methods [20]. They were cut into 5µm thick sections and stained using hematoxylin-eosin dye and finally mounted in di-phenyl xylene. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

2.10 Data Analysis

Data analyses were done using the GraphPad prism computer software version 5. Students't'-test and one-way analysis of variance (ANOVA) were used for comparison. A *P*-value < 0.05 was considered significant. The mathematical model was analysed using Maple 18.0 software.

3. RESULTS

3.1 Phytochemical Screening of Methanolic Leaf Extract of *Moringa oleifera*

Phytochemical screening of methanolic leaf extract of *Moringa oleifera* shows the presence of secondary metabolite like tannins, anthocyanine, steroid, anthraquinones, terpenoids and saponin (Table 1). The presence of these secondary metabolites in *Moringa oleifera* may be responsible for the hepatoprotective effect of the plant.

Table 1. Phytochemical screening of methanolic leaf extract of *Moringa oleifera*

Phytochemical constituent	Inference
Tannin	+++
Saponin	++
Antraquinone	+
Alkaloid	++
Steroid	+
Terpenoid	+
Phenolic compound	+
Anthocyanine	++
Reducing sugar	+
Resin	+

(+) present at low level, (++) present at moderate level, (+++) present at high level

3.2 Acute Toxicity Test

The oral administration of single dose of *Moringa oleifera* leaf extract (10, 100 and 1000 mg/Kg B.WT) in acute toxicity study showed no toxic sign or death of rats after one day. The LD_{50} value was calculated to be greater than 1000 mg/Kg body weight.

3.3 Hematological Analysis

Table 2 shows that there were significant increase (P<0.05) in WBC, HGB, RBC, HCT and PLT in the animals treated with *Moringa oleifera* leaf extract and standard drug compared to the

animals administered with CCl_4 without treatment.

3.4 Analysis of Liver Biomarker Enzymes and Lipid Profile

There were significant (P<0.05) increase in liver biomarker enzymes (AST, ALT, ALP and GGT) and lipid profiles (TC and TG) in group B untreated animals compared to all other groups (Table 3). They also have lower level of TP value.

3.5 Determination of Oxidative Stress Parameters

Oxidative stress parameters (SOD % inhibition, SOD unit, CAT, GSH and GPx) were significantly (P<0.05) reduced in animals administered with CCl_4 without treatment compared to the control group and treated groups respectively. Their MDA values (group B) were significantly (P<0.05) increased compared to other groups (Table 4).

Table 2. Hematological parameters of CCl_4 – induced hepatotoxic rats treated with silymarin, olive oil and *Moringa oleifera* extracts

Hematological parameters	Group A	Group B	Group C	Group D	Group E	Group F
WBC (×10^9/L)	13.5±7***	10.8±3	12.3±3*	11.9±3	16..3±4**	15.5±6**
HGB g/dl	14.7±3***	11.9±3	15.2±2*	11.6±1	15.5±2**	14.6±3**
RBC (×10^9/L)	7.5±1***	5.7±1	7.8±1*	6.1±1	8.4±1**	7.8±2**
HCT%	49.6±9***	43.8±8	51.4±9*	44.2±3	53.3±5**	49.4±10**
MCVfl	66.3±6	63.7±5	66.5±7	64.2±3	64.1±6	64.2±6
MCH pg	19.9±2	19.1±1	19.6±2	19.0±1	18.5±2	17.9±2
MCHC g/dl	30.1±1	30.1±2	29.6±4	29.6±1	29.0±2	28.0±2
RDW-CV %	17.9±2	16.8±1	16.6±1	17.0±2	16.7±1	16.9±1
RDW-SD fl	39.5±3	36.3±4	37.1±5	37.1±2	35.8±5	36.5±3
PLT (×10^9/L)	504.0±87.97***	353.0±59.4	575.3±57.98*	347.0±124.30	573.0±213.80**	754.3±310.1**
MPV fl	8.2±1	8.1±1	8.0±1	8.1±1	8.0±1	7.3±1
PDW	16.3±1	16.3±3	16.3±1	16.1±2	16.6±1	16.0±4
PCT %	0.423±0.084	0.462±0.207	0.461±0.076	0.516±0.016	0.467±0.306	0.482±0.106

*The values are the Means ± SD for five rats in each group. Hemoglobin (HGB), Red blood count (RBC), Hematocrit (HCT), Mean cell volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red Blood Cell Distribution Width Coefficient of Variation (RDW-CV), Red Blood Cell Distribution Width Standard Deviation (RDW-SD), Platelet count (PLT), Mean platelet volume (MPV), platelet Distribution Width (PDW) and Plateletcrit (PCT). Values with different asterisk superscripts are significantly different. * indicate significant difference of silymarin treated animals (P < 0.05) compared with Group B animals.** indicate significant difference of Moringa oliefera treated animals (P< 0.05) compared with group B animals. *** indicate significant difference (P < 0.05) of control animals (group A) compared with group B animals*

Table 3. Effect of silymarin, olive oil and *Moringa oleifera* extracts on plasma liver biomarker enzymes, TC, TG and TP in CCl_4-induced hepatotoxic rats

S/N	Parameters	Group A	Group B	Group C	Group D	Group E	Group F
1	AST (U/L)	13.3±1*	72.2±10	17.7±4*	64.5±5	20.2±2*	19.8±3*
2	ALT (U/L)	9.1±3*	42.4±6	18.8±5*	38.5±4	8.2±1*	6.5±2*
3	ALP (U/L)	9.2±1*	18.4±3	7.6±1*	16.1±2	11.1±2*	8.3±2*
4	GGT (U/L)	1.93±0.1*	9.26±0.4	3.09±0.1*	7.02±0.2	3.47±0.1*	3.86±0.2*
5	TC (mg/dl)	[a]75.4±15	126.2±21	87.6±19**	113.2±11	72.4±17***	81.5±26***
6	TG (mg/dl)	84.5±9**	111.0±21	90.5±17**	99.5±13	81.5±12**	83.8±18**
7	TP (g/dl)	9.7±1.7	8.2±1.3	9.2±2.4	8.7±1.6	11.9±2.4***	12.7±3.3***

*The values are mean ± S.D, for five rats in each group. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT), Cholesterol (TC), Triglyceride (TG), total protein (TP). Values with different asterisk superscripts are significantly different. * indicate significant difference between group A, C, E and F animals (p < 0.0001) compared with Group B animals.** indicate significant difference of group A, C, E and F animals (P < 0.05) compared with group B animals. [a] indicate significant difference of group A animals (P < 0.0006) compared with Group B animals. *** indicate significant difference of Moringa oliefera treated animals (p < 0.05) compared with group B animals*

Table 4. Effect of silymarin, olive oil and *Moringa oleifera* extracts on oxidative stress parameters in CCl_4-induced rats

S/N	Oxidative stress parameters	Group A	Group B	Group C	Group D	Group E	Group F
1	LPO (x10^3 mM MDA/mg protein)	9.86±1.9*	18.56±3.5	9.63±1.2*	17.59±1.1	7.17±0.9*	3.64±1.5*
2	CAT (µmol/min/mg protein)	73.82±21.8*	40.13±11.9	74.36±19.3*	49.30±11.4	74.54±17.3*	75.98±27.3*
3	SOD % inhibition	92.6*	56.5	94.6*	57.7	89.5*	90.4*
4	SOD unit	11.5±1.8*	1.27±0.6	17.76±3.5*	2.67±1.8	8.09±1.6*	9.61±2.1*
5	GSH (mg/mg protein)	0.35±0.06**	0.12±0.07	0.28±0.03**	0.16±0.03	0.27±0.05**	0.29±0.08**
6	GPx(min/mg protein)	9.23±1.7**	2.32±0.2	8.13±0.8**	2.92±1.1	7.23±0.8**	7.67±1.1**

*The values are mean ± S.D (n = 5). Lipid Peroxidative (LPO), catalase (CAT), superoxide dismutase % inhibition (SOD), SOD unit, reduced glutathione (GSH), Glutathione peroxidase (GPx). Values with different asterisk superscripts are significantly different. * indicate significant difference between group A, C, E and F animals (p < 0.0001) compared with Group B animals. ** indicate significant difference of group A, C, E and F animals (P < 0.05) compared with group B animals*

3.6 Mathematical Formulation and Model Description

The model consists of uninfected liver cells $x(t)$, infected cells $y(t)$ and free CCl_4 concentration $z(t)$ respectively. Uninfected cells are produced at a rate Λ and die out at a rate dx. Uninfected liver cells become infected at a constant rate β as a result of contact with CCl_4. Infected cells are produced at a rate βxz and die out at a constant rate α. Free CCl_4 concentration are introduced at a rate k and are removed from the system at a rate μ, k_1 represent the efficacy of the drug; Moringa. Thus, the following system of first order ordinary differential equations describes the foregoing discussion:

$$\left.\begin{aligned} \frac{dx}{dt} &= \Lambda - \beta xz - dx \\ \frac{dy}{dt} &= \beta xz - \alpha y \\ \frac{dz}{dt} &= k(1-k_1)y - \mu z \end{aligned}\right\} \quad (1)$$

3.6.1 The existence of the equilibrium state of the model

From system (1)

Set, $\frac{dx}{dt} = \frac{dy}{dt} = \frac{dz}{dt} = 0$

Hence, Infection–free equilibrium $\boldsymbol{\varepsilon_o} = \left(\frac{\Lambda}{d}, \mathbf{0}, \mathbf{0}\right)$ (2)

and the endemic equilibrium:

$$\varepsilon^* = (x^*, y^*, z^*) = \left(\frac{\Lambda}{dR_0}, \frac{\Lambda(R_0-1)}{\alpha R_0}, \frac{d(R_0-1)}{\beta}\right). \quad (3)$$

3.6.2 The basic reproduction number

The basic reproduction number R_0 is defined as the effective number of secondary infections caused by a single infected cell during its entire period of infectiousness [21]. The basic reproduction number is analyzed using the next generation matrix as described by [22] and [23] then R_0 Is the spectral radius (dominant Eigen-value) of FV^{-1} where F and V are the matrix of newly created infections and transferred infections. Thus,

$$F = \begin{pmatrix} 0 & \frac{\beta\Lambda}{d} \\ 0 & 0 \end{pmatrix}, V = \begin{pmatrix} \alpha & 0 \\ k(k_1-1) & \mu \end{pmatrix}$$

Thus the basic reproduction number for the model is

$$R_0 = \frac{\beta\Lambda(1-k_1)}{\alpha\mu d} \quad (4)$$

3.6.3 Local stability of the disease free equilibrium ε_o

Theorem 1: The disease free equilibrium $\boldsymbol{\varepsilon_o} = \left(\frac{\Lambda}{d}, \mathbf{0}, \mathbf{0}\right)$ of system (1) is locally asymptotically stable (LAS) if the basic reproduction number $R_0 < 1$, otherwise unstable.

Proof:

The Jacobian matrix of system (1) at the disease-free equilibrium point ε_O is

$$j(\boldsymbol{\varepsilon_o}) = \begin{pmatrix} -d & 0 & -\frac{\beta\Lambda}{d} \\ 0 & -\alpha & \frac{\beta\Lambda}{d} \\ 0 & k(1-k_1) & -\mu \end{pmatrix} \quad (5)$$

The eigen values of (5) are given by the following characteristic equation:

$$(-d-\lambda)f(\lambda) = 0 \quad (6)$$

Where

$$f(\lambda) = \lambda^2 + (\alpha+\mu)\lambda + \alpha\mu(1-R_0)$$

Clearly, $\lambda_1 = -d$ while the eigenvalues λ_2 and λ_3 of $f(\lambda)$ are real and negative if $R_0 < 1$ by Descartes rule of signs. Thus the effect of $CCl_4$was effectively reduced since the associated basic reproduction number$R_0 < 1$.Hence, the disease free equilibrium$\boldsymbol{\varepsilon_o} = \left(\frac{\Lambda}{d}, \mathbf{0}, \mathbf{0}\right)$ is locally asymptotically stable. (LAS)

3.6.4 The global stability of the disease free equilibrium (DFE) $\boldsymbol{\varepsilon_o}$

The method of Lyapunov function is used here to prove the global stability of DFE.

Theorem 2: The disease free equilibrium $\boldsymbol{\varepsilon_o} = \left(\frac{\Lambda}{d}, \mathbf{0}, \mathbf{0}\right)$ of system (1) is globally asymptotically stable (GAS) if the basic reproduction number $R_0 \le 1$ otherwise unstable.

Proof:

Let $L(y,z)$ defineaLyapunov function such that

$$L(y,z) = \alpha z + (1-k_1)ky \quad (7)$$

By differentiating (7) with respect to time t yield

$$\frac{dL(y,z)}{dt} = \alpha\frac{dz}{dt} + (1-k_1)k\frac{dy}{dt}$$

So that at the DFE

$$\frac{dL(y,z)}{dt} = \mu\alpha\left(\frac{\beta\Lambda k(1-k_1)}{\mu\alpha d} - 1\right)z$$

$$\frac{dL(y,z)}{dt} = \mu\alpha(R_0-1)z$$

Hence, $\frac{dL(y,z)}{dt} \le 0$ if $R_0 \le 1$ so that DFE is globally asymptotically stable

3.6.5 The local stability of the endemic equilibrium $\boldsymbol{\varepsilon}^*$

Theorem 3: The endemic equilibrium point $\boldsymbol{\varepsilon}^*$of system (1) is locally asymptotically stable if the following condition holds (i) $Tr(J(\boldsymbol{\varepsilon}^*)) < 0$ and (ii) $Det(J(\boldsymbol{\varepsilon}^*)) < 0$ whenever $R_0 > 1$ otherwise unstable.

Proof:

The Jacobian matrix of system (1) at $\boldsymbol{\varepsilon}^*$is

$$J(\boldsymbol{\varepsilon}^*) = \begin{pmatrix} -dR_0 & 0 & -\frac{\beta\Lambda}{dR_0} \\ d(R_0-1) & -\alpha & \frac{\beta\Lambda}{dR_0} \\ 0 & k(1-k_1) & -\mu \end{pmatrix} \quad (8)$$

The eigenvalues of $J(\boldsymbol{\varepsilon}^*)$ are all and negative if (i) $Tr(J(\boldsymbol{\varepsilon}^*)) < 0$ and (ii) $Det(J(\boldsymbol{\varepsilon}^*)) < 0$ whenever $R_0 > 1$

Simple calculation shows that

(i) $Tr(J(\boldsymbol{\varepsilon}^*)) = -dR_0 - \alpha - \mu$
$= -(dR_0 + \alpha + \mu) < 0$

(ii) $Det(J(\boldsymbol{\varepsilon}^*)) = -\frac{\beta\Lambda k(1-k_i)(R_0-1)}{R_0} < 0$ if $R_0 > 1$

Thus, the endemic equilibrium point of system (1) is locally asymptotically stable if $R_0 > 1$ otherwise unstable

3.6.6 The effect of drug therapy on the liver of the male albino rat

The basic reproduction number R_0 determine the dynamics of the disease progression or its eradication. Thus if

$$\frac{\partial R_0}{\partial k_i} = -\frac{\beta\Lambda k}{\mu\alpha d} \quad (9)$$

The negative sign at right hand side of (8) indicate the application of drug therapy (standard drug or Moringa Extract) will reduce the basic reproduction number and hence the infection will die out with time.

3.7 Histopathological Studies

The liver architecture of the healthy animal, animal infected with CCl_4 without treatment and animal treated with *Moringa oleifera* extract are shown in Figs. 5, 6 and 7 respectively.

4. DISCUSSION

The methanolic leaf extract of *Moringa oleifera* contain phytochemicals like: tannin, alkaloid, saponin, antraquinone, steroid, resin, reducing sugar, phenolic compound, anthocyanine and terpenoid (Table 1).

The acute toxicity study shows that the plant extract of *Moringa oleifera* is non- toxic and no mortality was observed in all the groups. The calculated LD_{50} value was greater than 1000 mg/Kg B.WT.

It is generally believed that the hepatotoxicity induced by CCl_4 is due to the formation of the active metabolite, trichloromethyl free radical (CCl_3•). This then readily interacts with molecular oxygen to form the trichloromethyl peroxy radical (CCl_3OO•). Both radicals are capable of binding to proteins and other macromolecules with simultaneous attack on poly-unsaturated fatty acids to produce lipid peroxidation leading to hepatotoxicity [24].

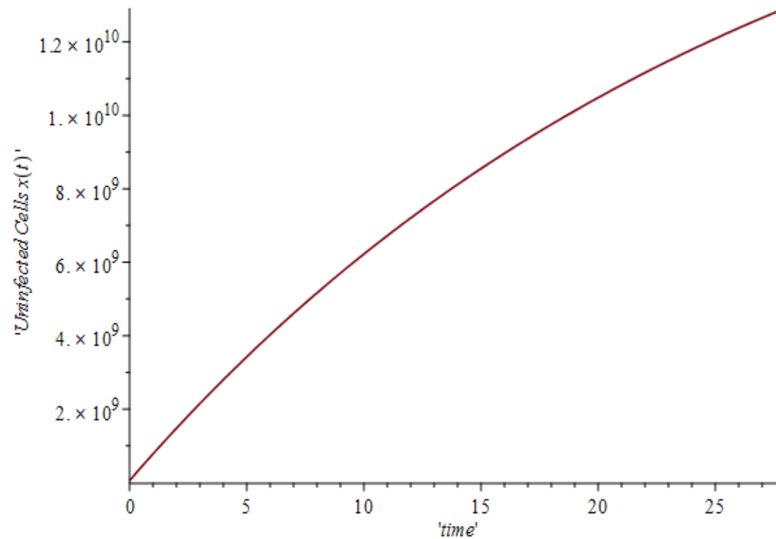

Fig. 1. The graph of unifected cells when the basic reproduction number is less than unity

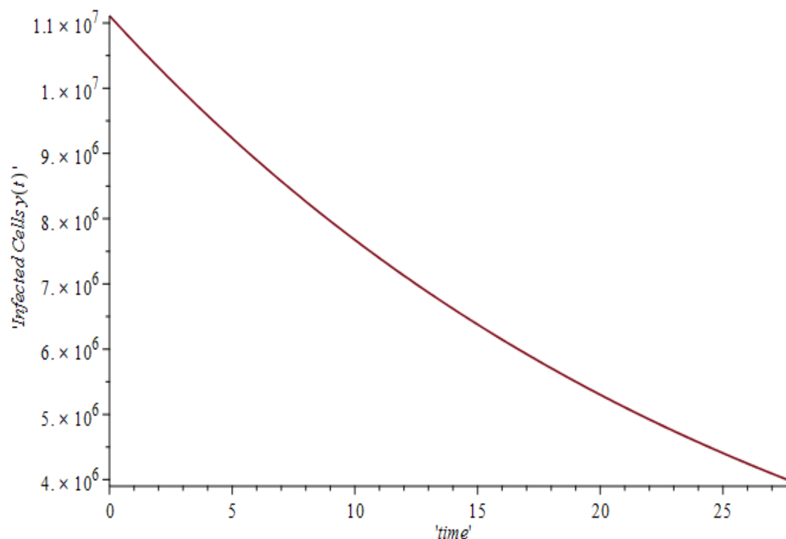

Fig. 2. The graph of infected cells when the basic reproduction number is less than unity

Hematological and biochemical indices are reliable parameter for the assessment of the health status of animals [25-26]. The primary reason for assessing the RBC is to check the level of anemia and to evaluate normal erythropoiesis. HGB level shows the amount of intracellular iron present while HCT indicates the volume of RBC in 100ml of blood and it helps to determine the degree of anemia or polycythemia.

WBC helps the body to fight infection, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least to transport and distribute antibodies in immune response. The study shows that there are significant decrease ($p<0.005$) in the level of blood WBC HGB, HCT and RBC of the CCl_4 intoxicated animals (Group B) compared to the animal treated with the *M. oleifera* extract (Table 2). The significant reduction ($P < 0.05$) in these hematological parameters in Group B animals may be attributed to the cytotoxic effects and suppression of the erythropoiesis caused by constituents of the CCl_4.

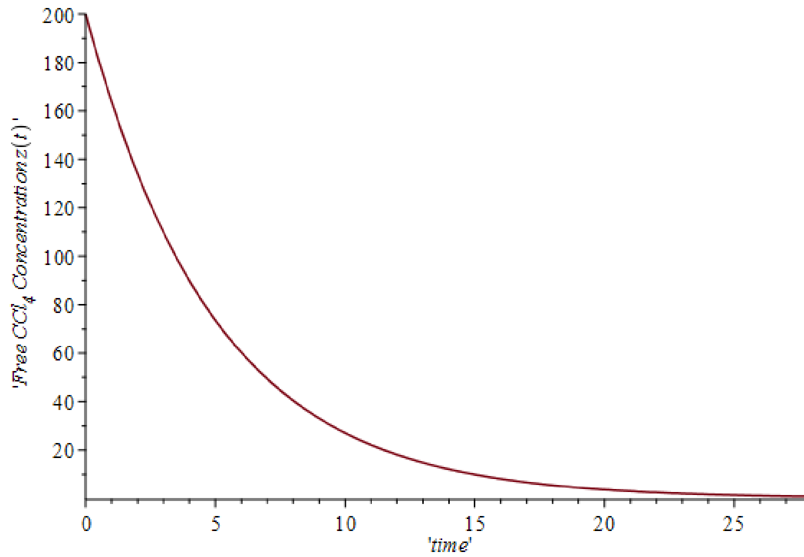

Fig. 3. The graph of z(t) when the basic reproduction number is less than unity

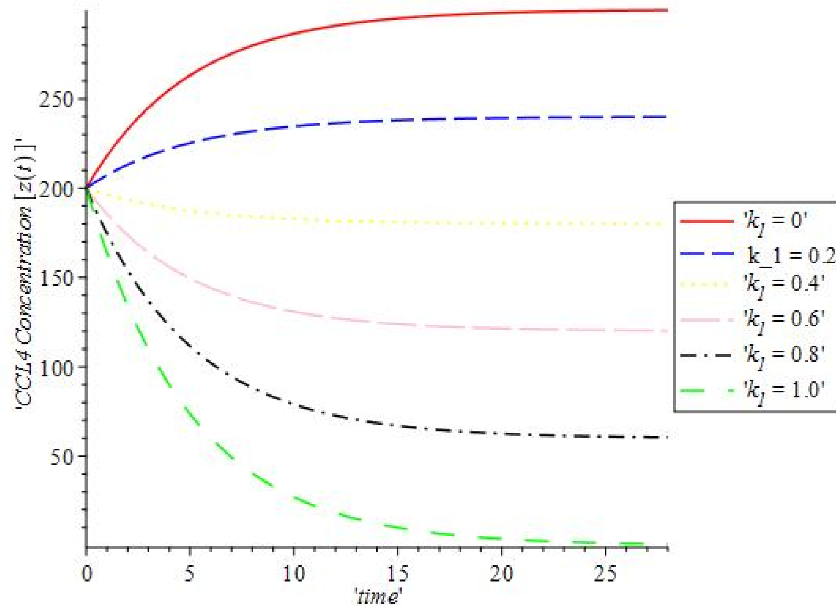

Fig. 4. The effect of *Moringa oleifera* on the CCL_4

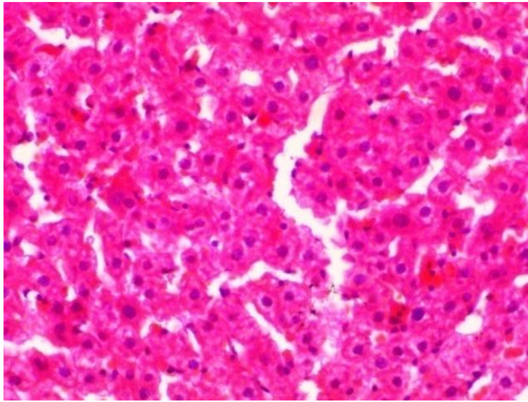

Fig. 5. Photomicrograph of liver sections stained with hematoxylin and eosin (H&E X 400) for control group showing the normal histological structure of hepatocytes, hepatic cords, central vein and sinusoids.

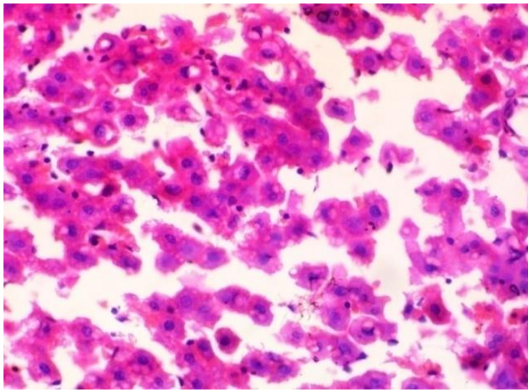

Fig. 6. Photomicrograph of liver sections stained with hematoxylin and eosin (H&E X 400) for rat treated with CCl_4 and olive oil (20% CCl_4 in olive oil)

There were significant increase (P<0.05) in the PLT number in the animals treated with silymarin and *M. oleifera* extract compared with animals administered with CCl_4 without treatment. Other hematological parameters like: PCT, MPV, PDW, MCH, MCHC, RDW-CV, RDW-SD and MCV showed no significant differences in the entire groups. The results obtained from this study showed clearly that methanolic leaf extracts of *Moringa oleifera* is not hematotoxic.

The result from the study showed that there were significant increase (P<0.0001) in the ALP and GGT values of group B rats compared to the healthy rats and the treated groups. This may imply that damage occur in the liver cells of the animals administered with CCl_4 since the activities of these enzymes are reported to be increased in liver damage. There was significant increase (P<0.05) in the plasma levels of AST and ALT (Table 3) values in animals of the CCl_4 intoxicated rats (group B) compared to the control group, animals treated with silymarin and *M. oleifera* leaf extracts respectively. These results are in agreement with those reported by Singh, Girish and Pradhan [27-28]. Treatment with silymarin and *Moringa oleifera* extracts markedly reduced the effect of CCl_4 induced liver damage as evidenced by decreased level of plasma activities of AST, ALT, ALP, GGT and reduced plasma concentration of TG and TC as shown in Table 3. These transaminases (AST and ALT) have high concentrations and can be liberated from the hepatocyte cytoplasm; they are sensitive indicators of necrotic lesions within the liver [29-30]. Hence, the marked release of transaminases into the blood circulation is an indication of severe damage to hepatic tissue membranes during CCl_4 intoxication [31-32]. The significant increase in these liver biomarker enzymes (AST, ALT, ALP and GGT) in the plasma of these animals is an indication of the hepatotoxicity of the liver in the animals administered with CCl_4 as described by Mahesh *et al* 2009 [29] and this causes cellular leakage and loss of functional integrity of the hepatic cell membrane [33-34]. We observed significant decrease (P <0.0001) in plasma transaminase activities in the animals in group A, silymarin, and *M. oleifera* extracts treated groups and these indicate their hepatoprotective effects against CCl_4 damage. The significant decrease (P<0.05) in the total protein values of animals administered with CCl_4 without treatment compared to healthy animals may causes considerable liver damage through induction of peroxidation of lipids and finally inhibits the protein synthesis due to trichloromethyl free radical covalent bindings [35]. The treatment with *M. oleifera* extract and silymarin stabilized the plasma total protein. The stabilization of proteins might be considered as an indication of enhanced protein synthesis in the hepatic cells due to inhibition of peroxidation of lipids and scavenging of free radicals [36].

Oxidative stress is caused by the presence of reactive oxygen species (ROS) in excess of the available of antioxidant buffering capacity. Many studies have showed that ROS can damage lipids, proteins and DNA, thus altering the structure and function of the cell, tissue, organ and system respectively. The antioxidants could attenuate this oxidative damage caused by free

radicals indirectly by enhancing natural defenses of cell and/or directly by scavenging the free radicals [37].

The antioxidant defense mechanisms include enzymatic and non-enzymatic antioxidants playing a significant role in sustaining physiological levels of O_2 and H_2O_2 and eradicating the peroxides generated from inadvertent exposure to toxic drugs. Any natural medications with antioxidant profiles may help to maintain health when continuously taken as components of dietary food, spices or remedies [38].

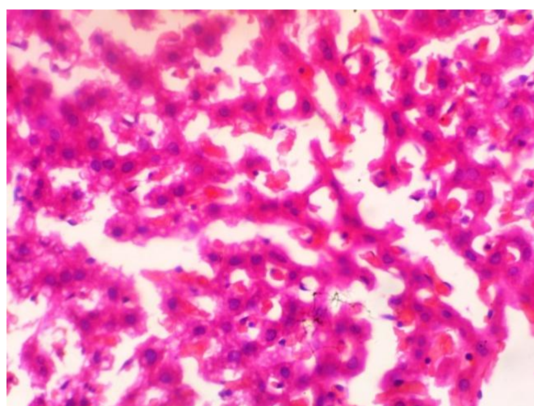

Fig. 7. Photomicrograph of liver section stained with hematoxylin and eosin (H&E X 400) for rat treated with *Moringa oleifera* leaf extract daily for 28 days and received CCl_4 – olive oil mixture (20% CCl_4 in olive oil) twice a week for four weeks

SOD is an effective defence enzyme that catalyses the dismutation of superoxide anions into hydrogen peroxide. Catalase catalyses the conversion of hydrogen peroxides into oxygen and water and protects the tissue from oxidative damage by highly reactive oxygen free radicals and hydroxyl radicals [39]. GSH acts as a non-enzymatic antioxidant that reduces hydroperoxides (ROOH) and xenobiotic toxicity. Lipid peroxides or other ROS easily inactivate these antioxidant enzymes, which results in reduced activities of these enzymes in CCl_4 toxicity. In this study, there was significant increase ($P<0.05$) in the SOD % inhibition, SOD unit, reduced glutathione, glutathione peroxidase and catalase in liver tissue homogenate of the rats treated with silymarin, *Moringa oleifera* leaf extracts and group A animals when compared with group B animals. After the CCl_4 induced hepatotoxicity, GPx along with catalase metabolize H_2O_2 to water and other non-toxic substances. This antioxidant system also consists of GSH, a range of functionally interrelated enzymes and GPx that are responsible for the regeneration of GSH or from GSSG to GSH, where both enzymes work together with GSH in the decomposition of hydrogen peroxide and also other biological hydroperoxides [40]. The dose-dependent increase of GPx activity with the *M. oleifera* extract treatment showed the antioxidant activity of plant extract by scavenging the endogenous metabolic peroxides generated after CCl_4 induced damage in the liver tissue. MDA increased after oral administration with CCl_4, treatment with *Moringa oleifera* leaf extracts and silymarin reduce the level of MDA ($P<0.0001$). Inhibition of elevated LPO has been observed in *M. oleifera* extract and silymarin treated groups due to its antioxidant and free radical scavenging activities through re-establishment of biomembranes of hepatic parenchymal cells [41]. Liver tissue contains relatively high content of polyunsaturated fatty acids (PUFAs), which are sensitive to peroxidative damage [42-43] and lead to an increase in lipid peroxidation in the group of animals intoxicated with CCl_4 without treatment compared to other treated groups. The observed hepatoprotective effect of silymarin against lipid peroxidation could be related to its antioxidant effects which assist in the preservation of membrane integrity. Silymarin can chelate transition metal ions such as iron and copper, rendering them effective antioxidants [44]. Increased lipid peroxidation, as evidenced by the elevated levels of MDA in hepatic tissues demonstrated in this study after administration of CCl_4 could be expected owing to the depletion in GSH concentration.

Fig. 1 shows the increase in the number of uninfected cell when the basic reproduction number is brought below unity. This result is in perfect agreement with our experimental result. The efficacy of the *M. oleifera* is clearly seen in Fig. 1 where the number of infected cells decreases significantly, a result which also agrees with our experimental result. Figs. 2 and 3 underline the efficacy of *M. oleifera* extracts in reducing the effect of CCl_4 concentration based on twenty eight (28) days of treatment and observation, the *M. oleifera.* extract prove to be efficient in reducing the damage caused by CCl_4. Fig. 3 specifically show that if the efficacy of the therapy is zero, the damage caused by the CCl_4 increases. However, if the efficacy of the *M. oleifera* therapy is one, the effect of the

damage done will be significantly reduced resulting in increase in number of healthy (uninfected) cells as shown in Fig. 1 with time.

Histopathological study also provides important evidence supporting the biochemical analysis of the liver. The liver tissue of control rat showed normal histological structure of hepatocytes, hepatic cells with well preserved cytoplasm as shown in Fig. 5. The administration of CCl_4 showed harmful effects on liver tissues (Fig. 6) and this include: fatty changes of hepatocytes, severe histopathological damage in the liver with significant degeneration of cells, severe hepatocyte necrosis and inflammatory cell infiltration. The damage extended to the majority of the hepatic lobule with marked loss of its normal pattern. These changes positively correlated with the noted increase in transaminase activities (AST, ALT, ALP and GGT) in CCl_4-induced hepatotoxicity. The animals treated with the extract (Fig. 7) were observed to show improved generation of cells, moderate degrees of fibrosis, milder degree of hepatocyte necrosis and they gave a better histopathological result when compared to the animal administered with CCl_4 without treatment.

5. CONCLUSION

In conclusion, the results of this study demonstrate that silymarin and *Moringa oleifera* extract were effective in reducing the hepatic damage caused by CCl_4 in Sprague Dawley male albino rats.

CONSENT

This was not applicable since the study was on animals and not on humans.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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