NEXUS OF MEDICINE AND LABORATORY SCIENCE JOURNAL

ISSN (ONLINE): 3027-2998

Remedial Effect of *Mormodica Charantia* on Hepatic Inflammation and Oxidative Stress Induced by Carbon Tetrachloride in Wistar Rats

Bini, N[1]*, Osadolor, H. B[2]; Elekofehinti, O. O[3], Dic-Ijiewere, E. O[4]

[1]* Department of Medical Laboratory Science, University of Delta, Agbor. Delta State, Nigeria

[2] Department of Medical Laboratory Science, University of Benin, Benin City. Nigeria

[3] Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria

[4] Department of Chemical Pathology, Faculty of Medical Laboratory Science, Ambrose Ali University, Ekpoma, Edo State, Nigeria

*Correspondence Author's email contact: nathaniel.bini@unidel.edu.ng; +2348026174400

Article type: Original

Cite as: Bini N, Osadolor HB, Elekofehinti OO, Dic-Ijiewere EO. Remedial Effect of Mormodica Charantia on Hepatic Inflammation and Oxidative Stress Induced by Carbon Tetrachloride in Wistar Rats. Nexus Med. Lab. Sci. J. 2025;2(2):1-22

Received on 6th March, 2025; Accepted on 2nd May, 2025; Published on 14th May, 2025

Publisher: ScholarlyFeed

https://doi.org/10.71462/sfpl2504004

Abstract

Background: Anti-inflammatory and antioxidant properties of *Mormodica charantia* has been a crucial factor in reducing liver damage. The medicinal value of *M. charantia* is derived from its bioactive components, which have observable physiological effects on the body and act as a preventive measure. The present study aimed at evaluating the antioxidant and anti inflammatory effects of *M. charantia* on acute liver injury induced by carbon tetrachloride (CCL_4) in wistar rats.

Method: Wistar rats were subjected to intraperitoneal injection of 1 ml/kg body weight CCL_4 with or without *M. charania* (100 mg/kg, 200 mg/kg or 300 mg/kg). Rats were randomly divided into six (6) groups, comprising five rats each. The treatment schedule was as follows; Group I: Normal Control; Group II: Negative Control (CCL4 only); Group III:CCL4 + *M. charantia* (100 mg/kg); Group IV:CCL4 + *M. charantia* (200 mg/kg); Group V:CCL4 + *M. charantia* (300 mg/kg); and Group VI: CCL4 + Silybon (40 mg/kg). The treatment commenced after CCL4 injection.

Results: Rats treated with CCL_4 developed acute liver injury, as evidenced by elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Furthermore, increase in white blood cells (WBC), platelet counts (PLT) and pro- inflammatory cytokines; tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin 6 (IL-6), alongside decrease in haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), antioxidant genes; superoxide dismutase (SOD), catalase (CAT), aryl hydrocarbon receptor (AhR) and NAD(P)H quinone oxidoreductase 1 (NQ01), and anti-inflammatory gene; interleukin 10 (IL-10), were observed. *M. charantia* treatment significantly and dose- dependently reduced liver injury, oxidative stress and inflammation. Inflammatory cytokines were downregulated by *M. charantia* and CCL_4- induced oxidative stress was blocked by M. *charantia* treatment through improving anti- oxidants. The changes was also evidenced by histological analysis.

Conlusion: The present study highlighted that *M. charantia* exhibited liver- protective effects against acute hepatic injury induced by CCL_4 via suppressing inflammation and oxidative stress.

Key words*: Mormodica charantia*, liver injury, oxidative stress.

Introduction

The liver is an important organ that plays important roles in the body including detoxification of harmful substances, metabolism of macromolecules, synthesis of plasma protein and regulation of body glucose level [1, 2]. The liver is made up of a variety of cell types, all of which communicate with one another in order to carry out their respective duties. Hepatocytes, also known as hepatic parenchymal cells, are responsible for up to 80 percent of the organ's overall volume, and they perform the majority of the liver's tasks [1]. Liver diseases are responsible for the deaths of around 2 million people per year all over the world [3, 4]. Excessive consumption of alcohol, viral infection, blockage of the biliary system, exposure to heavy metals are all risk factors of liver damage [5, 6]

Carbon tetrachloride (CCl_4), is one of the toxins that is utilized most frequently in the context of experimental research on liver diseases. The liver is a large organ with specialized metabolic activities and the principal target of CCl_4 poisoning. CCI_4 transfers an electron from the C–Cl link, forming a radical anion that breaks down into trichloromethyl (CCl3) and chloride [7, 8]. Trichloromethyl may transform into chloroform, attach to biological components, and damage cell membrane fatty acids. Chloroform and secondary lipid radicals react with molecular oxygen to form lipid peroxyradicals. The trichloromethyl radical can interact with oxygen to generate the peroxytrichloromethyl free radical, which is more reactive but causes equivalent damage. Peroxidized fatty acids break down into carbonyls such as malondialdehyde (MDA), ethane, and pentane [7, 9, 10].

Mechanisms through which CCl_4 induced hepatotoxicity includes generation of free radical and associated reactive species, Kupffer cells activation, decrease in the content of 5-methylcytosine in the genome, as well as the production of cytokines and interleukins [11, 12]. As a result, the hepatocytes become more susceptible to oxidative stress. Excessive free radical production is a major contributor to liver damage caused by excessive oxidative stress. On the other hand, CCl4 decreases the antioxidant enzyme activities of the liver cells, such as catalase, superoxide dismutase (SOD) [13]. The activation of Kupffer cells following liver injury leads to the production of cytokines and interleukins such as IL-6, IL-10, IL-1β and TNF-α [12, 14, 15]. The liver's serum marker enzyme concentrations are markedly elevated after CCl4 administration. It increases the levels of serum enzymes like Alanine Transaminase (ALT) and Aspartate Transaminase (AST) that are typically present in the cytoplasm [13, 16].

Despite significant advances in medicine, there is no effective treatment that stimulates liver functions, protects the liver from injury and aids in the regeneration of hepatic cells [17,18]. Silybon commonly used to aid in the management of liver damage caused by chronic liver disease and cirrhosis. It is also known for protecting the liver from damage caused by chemicals, alcohol, chemotherapy, nonalcoholic fatty liver disease, and chronic hepatitis [19a]. As an alternative to or supplement to conventional treatment, herbal medicines have garnered significant attention, and the need for these cures is now on the rise [19]. Studies have shown that *M. charantia* has diverse pharmacological activities including wound healing properties owing to its powerful antioxidant properties [20, 21]. Despite the enriched pharmacological properties of *M. charantia*, there its dearth of information on its protective function in liver injury. Hence, this study investigates the antioxidant and anti-

inflammatory effect of *M. charantia* on CCL_4-induced liver injury in wistar rats.

Materials and methods

Experimental design

Thirty (30) male wistar rats weighing approximately 250-300 grams were purchased from the Department of Biochemistry, Federal University of Technology Akure. The rats were kept in the department animal house, and they were provided with rat normal pellet diet and water *ad libitum*. The rats were acclimatized for 14 days

Induction of liver injury

The experimental animals were given by an intraperitoneal injection of CCl_4 (1 mL/kg.b.wt) to induce liver injury, as previously described by [22]. Rats were randomly divided into six (6) groups, comprising five rats each. The treatment schedule was as follows:

Group I: Normal Control; Group II: Negative Control (CCL4 only); Group III:CCL4 + *M. charantia*(100 mg/kg); Group IV:CCL4 + *M. charantia*(200 mg/kg); Group V:CCL4 + *M. charantia*(300 mg/kg); and Group VI: CCL4 + Silybon (40 mg/kg). The treatment commenced after CCL4 injection just one-off time. The experiment lasted for 28 days while the weights of the rat were recorded periodically. At the end of the management period, each rat was anesthetized by diethyl ether. Blood samples via cardiac puncture were collected for biochemical . (ALT, AST, ALP) and haematological analysis (Hb, RBC, PCV). Using scissors and forceps, liver tissues were removed, trimmed of fatty tissue, rinsed in normal saline (0.9% NaCl), and weighed. Using a Teflon homogenizer, 100 mg of the tissues were homogenized in 1 ml of normal saline per milligram of tissue. The resultant homogenates were centrifuged at 300 rpm and 40 degrees Celsius before being utilized in biochemical tests. (TNF-α, IL-1β, IL-6, SOD, CAT, AhR, NAD(P)H, NQ01), and IL-10),

Collection of plant material

The leaves of *M. charantia* was cultivated within the University of Benin Main Campus, the plant material was taken to faculty of life science, University of Benin for authentication. The *M. charantia* leaves were rinsed with distilled water and air dried for a duration of 17 days.

Extraction of *M. charantia* leaves

The air-dried leaves of *M. charantia* was pulverized into smooth and fine particles using an electric blender. Five-hundred and thirty grams of the powered leaves (530 g) was weighed using a weighing balance, and was added to 1590 ml of methanol. The mixtures were allowed to stand for 48 h, during which it was stirred periodically. The mixtures were sieved using filter paper to obtain the mixture filtrate. The filtrate was taken to the Department of Medical Biochemistry, University of Benin for freeze-drying. The filtrate was freeze dried to obtain methanol extract of *M. charantia*. The extract was stored at room temperature (25^{o}C).

Estimation of liver enzymes assays

The activities of aspartate aminotransferase (AST) and level of total protein was determined based on Bradford method [23] using commercially available kit (Agappe). The level of alanine aminotransferase (ALT) level was determined based on the method of Reitman and Frankel [24] using commercially available kit (Randox laboratories UK. Alkaline Phosphatase (ALP) level was determined based on the method of [25] using commercially available kit (Randox laboratories, UK).

Determination of hematological parameters

The haematology parameters were analyzed using five (5) part Mindray Haematology Aanalyser (Mindray BC-500) using Coulter principle.

Principle

The Coulter method of sizing and countering particles is based on measurable changes in electrical impedance produced by nonconductive particle suspended in an electrolyte. [26]

Gene expression assay

RNA isolation and reverse transcription

The excised liver tissues were homogenized using mortal and pestle to increase the surface area of the tissues. The homogenized tissues were placed in eppendoff tubes containing some quantity of Trizol reagents. The homogenate was centrifuged for 30 minutes at 1500 rpm after being treated with a gradient separation medium (chloroform). To recover the RNA pellet, the supernatant was mixed with precipitating media (isoamyl alcohol) and vortexed at 1500 rpm for 30 minutes. After that, the supernatant was decanted. In nuclease-free water, the precipitated RNA was suspended. Following that, the total RNA concentration was determined using UV absorbance spectrophotometry (JENWAY 6305). The total RNA yield was converted to cDNA using reverse transcriptase enzyme, which is available in a protoscriptll cDNA synthesis kit.

Amplification of genes using polymerase chain reaction

The synthesized cDNA was aliquoted into a primer-specific PCR cocktail and diluted in Nuclease-free water. In brief, the cocktail contains some quantity (µL) of reverse and forward primers, synthesized cDNA, master mix. The cocktails were loaded on thermocycler to carry out the amplification of the genes under 30 PCR amplification cycles. Typically, a GAPDH-specific primer was used to track each sample animal's baseline gene expression. The primer sequences of the amplified genes are: aryl hydrocarbon receptor (AHR) forward primer 5'-CAGGCGTTCCTAAGCAAGTTTC -3', reverse primer 3'-GGAGGTGAGCAGCAGTCTGA-5'; Catalase (CAT) forward primer 5'-GATGGTAACTGGGACCTTGTG -3', reverse primer 3'-GTGGGTTTCTCTTCTGGCTATG -5'; IL-1B forward primers 5'-GCAATGGTCGGGACATAGTT -3', reverse primer 3;-AGACCTGACTTGGCAGAGGA -5'; IL-6 forward primer 5'-TCTCTCCGCAAGAGACTTCCA -3', reverse primer 3'- ATACTGGTCTGTTGTGGGTGG-5'; IL-10 forward primer 5'-GAGAGAAGCTGAAGACCCTCTG -3', reverse primer 3'-TCATTCATGGCCTTGTAGACAC-5'; quinone oxidoreductase 1(NQO1) forward primer 5'-CAGCGGCTCCATGTACT – 3', reverse primer 3'-GACCTGGAAGCCACAGAAG -5'; SOD forward primer 5'-AGGGCCTGTCCCATGATGTC -3', reverse primer 3'-AGAAACCCGTTTGCCTCTACTGAA -5'; TNF-alpha forward primer 5'-ACCACGCTCTTCTGTCTACTG -3', reverse primer 3'-CTTGGTGGTTTGCTACGAC-5'; GAPDH forward primer 5'-AGACAGCCGCATCTTCTTGT -3', reverse primer 3'-CTTGCCGTGGGTAGAGTCAT -5'.

Agarose gel electrophoresis

The PCR amplicons were subjected to agarose gel electrophoresis. The migration of the DNA bands was visualized under blue light imager. The relative density of the DNA bands was captured via snapshots. Subsequently, the densitometric values of the bands were quantified using Image J software (http://imagej.en.softonic.com).

Histopathological examination

The liver tissues were divided into aliquots and fixed in 10% formalin in phosphate-buffered normal saline for a week. After being rinsed for two hours under running water, they underwent progressive ethanol dehydration before being embedded in paraffin wax. After that, the sections were xylene deparaffinized and hematoxylin and eosin stained. With the use of x40 and x100 objective lenses, the created tissue slices were viewed, photographed, and evaluated under a microscope [4].

Statistical analysis

GraphPad 8 Software was used to analyze the data using one-way analysis of variance (ANOVA) (USA). The findings were presented as mean±SD. We made numerous comparisons between the two groups using the Bonferroni post-hoc test. When the p-value was less than 0.05, the differences were determined to be statistically significant.

RESULTS

Effect of *M.charantia* on the bodyweight of CCl_4-induced liver injury in wistar rats

As shown in Table 1, induction of liver injury following intraperitoneal injection of CCl_4 caused significant reduction ($p<0.05$) in the weight of the wistar rats which signifies the onset of hepatic injury. Oral administration of 200 mg/kg and 300 mg/kg of *M. charantia* to CCl_4-induced rats caused significant increase in the weight of the rats when compared to *negative control*, in second and third week of treatment. On the 4th week of treatment, there was significant increase in the weight of CCl_4 induced rats in CCL4 + M. C (200 mg/kg), CCL4 + M. C (300 mg/kg), and CCL4 + Silybon (40 mg/kg) treatment groups. It should be noted that treatment of CCl_4-induced rats with silybon (40 mg/kg) did not experience a significant increase in the rats' weight when compared with negative control group until third week of treatment. Also, there was no significant difference ($p<0.05$) in the weight of CCl_4-induced rats when compared to negative control throughout the duration of the treatment.

Table 1: Effects of *Mormodica charantia* on CCL4 induced changes in body weight (g) of wistar rats

Treatment	Before Induction (g)	After induction (g)	Week 1 treatment (g)	Week 2 treatment (g)	Week 3 treatment (g)	Week 4 treatment (g)
Control	195.44±2.10	194.87± 2.10	195.36± 3.12^{d}	192.88±3.11	198.11± 3.23	201.48±3.15
-ve control	200±1.53	173.22±3.35^{b}	167.40± 2.56	165.93±5.01^{b}	166.15± 5.67^{b}	165.53±4.55^{b}
CCL_4 + M.C (100mg/kg)	192±4.05	163.93± 2.74^{b}	168.74± 3.08^{b}	164.26± 3.11^{b}	165.48± 3.10^{b}	168.86± 3.23^{b}
CCL_4 + M.C (200mg/kg)	196±4.31	170.74± 2.83^{b}	179.35± 3.08^{bdf}	176.88± 3.10^{bdf}	182.09± 4.04^{bdf}	185.48± 3.97^{bdf}
CCL_4 + M.C (300mg/kg)	198±4.11	174.19± 3.12^{b}	187.61± 3.08^{adf}	185.13± 3.10^{adf}	190.36± 3.10^{bdf}	193.73± 3.11^{df}
CCL_4 + Silybon (40mg/kg)	194±2.93	162.43± 2.88^{b}	168.43± 3.20^{b}	165.95± 3.27^{b}	171.18± 3.27^{bc}	174.55± 3.27^{bd}

"a" represent $p<0.01$ to Negative control, "b" represents $p < 0.01$ to control, "c" represents $p < 0.05$ to Negative control, "d" represents $p < 0.01$ to Negative control, and "f" represents $p < 0.01$ to CCL4 + Silybon (40 mg/kg).

Table 2 shows the effects of *M. charantia* on the blood parameters of CCl_4-exposed wistar rats. The result showed a significant ($p<0.05$) decrease in the level of Hb and RBC count in the negative control group compared to the control group. There is significant ($p<0.05$) increase in the levels of Hb and RBC count in the *M. charantia* and silybon treatment groups compared to the negative control group. Also, there is a significant ($p<0.05$) decrease in the levels of Hb and RBC count in the (100 mg/kg) and (200 mg/kg) *M. charantia* treatment groups but a significant ($p<0.05$) decrease in the levels of Hb and RBC count in the (300 mg/kg) *M. charantia* treatment group when compared with the silybon treatment group. The results also show that there is significant ($p<0.01$) increase in the WBC and PLT counts but a significant ($p<0.01$) decrease in the PCV percentage of the negative control group compared to the control group. Treatment with *M. charantia* at (100 mg/kg), (200 mg/kg) and (300 mg/kg) as well as silybon at (40 mg/kg) was able to significantly ($p<0.05$) decrease the count of RBC

and PLT and significantly ($p<0.05$) increase the PCV percentage of the treatment groups compared to the negative control group.

Table 2: Effect of *M. charantia* on CCl4 induced changes in haematology parameters of the Wistar rat

Treatment	RBC ($x10^6$//µL)	Hb(g/dl)	WBC($x10^3$)	PCV (%)	PLT($x10^3$ /µL)
Control	7.87±0.16	15.13±0.29	6.18±0.34	46.17±0.83	172.33±23.61
-ve Control	5.06±0.16[b]	10.84±0.39[b]	9.46±1.32[b]	26.40±1.03[b]	114.60±27.48[b]
CCL_4 + M.C (100mg/kg)	5.48±1.12[df]	11.10±2.44[df]	8.55±1.51[df]	37.00±7.47[df]	129.33±28.69[cf]
CCL_4 + M.C (200mg/kg)	6.83±0.03[df]	13.20±0.30[df]	6.00±0.15[d]	46.00±1.16[de]	137.00±19.04[df]
CCL_4 + M.C (300mg/kg)	8.36±1.28[df]	15.12±2.63[de]	5.57±1.35[de]	48.00±8.41[de]	171.67±31.99[de]
CCL4+Silybon (40mg/kg)	7.36±1.34[d]	14.86±2.73[d]	6.04±1.93[d]	45.20±9.08[d]	169.80±45.58[d]

"b" represents $p < 0.01$ to control, "c" represents $p < 0.05$ to Negative control, "d" represents $p < 0.01$ to Negative control, "e" represents $p < 0.01$ to Negative control and "f" represents $p < 0.01$ to CCL4 + Silybon (40 mg/kg).

Effect of *M. charantia* on the liver function indices of CCl_4-induced liver injury in wistar rats

Figure 1a, **1b**, **1c**, **1d**, and **1e** show the effect of *M. charantia* extract on liver AST, ALT and ALP in CCl_4-induced liver injury in wistar rats. There was significant increase ($p<0.05$) in the level of AST ALT and ALP in negative control when compared to the control group. There was significant reduction in the level of AST, ALT and ALP following administration of vary dose of M. charantia (100 mg/kg, 200 mg and 300 mg/kg) respectively and Silybon when compared with negative control. However, there was significant reduction ($p<0.01$) in the level of AST and ALP following treatment with 100mg/kg and 200 mg/kg of M. charantia when compared with CCl_4 + silybon group.

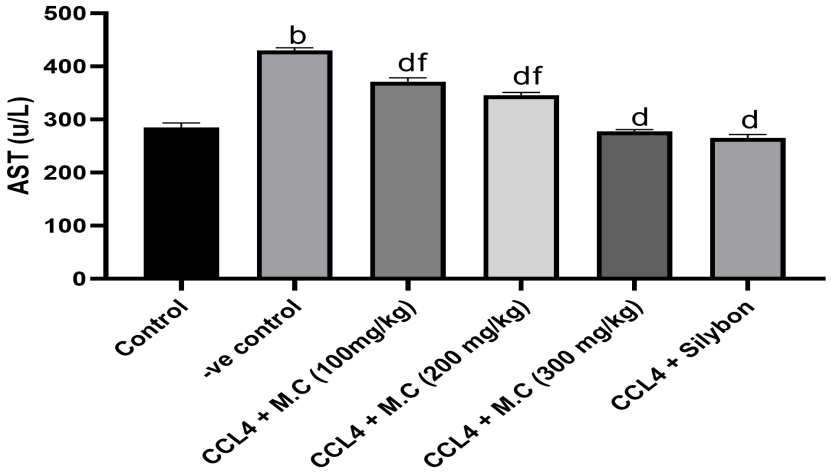


Figure 1a: The effect of *M. charantia* extract on the level of liver aspartate aminotransferase in CCl_4-induced liver injury in wistar rats. "b" represents $p < 0.01$ to control, "d" represents $p < 0.01$ to Negative control, "f" represents $p < 0.01$ to CCL4 + Silybon (40 mg/kg).

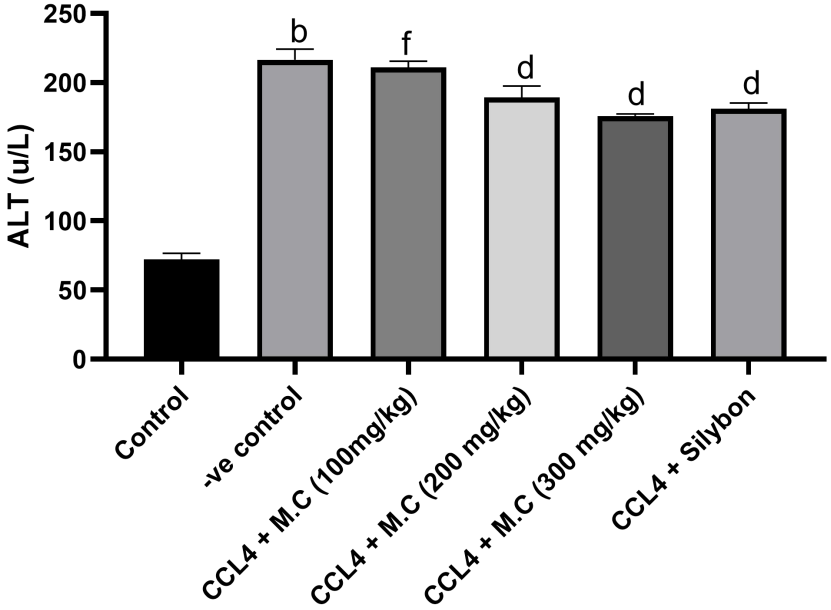


Figure 1b: The effect of *M. charantia* extract on the level of liver alanine aminotransferase in CCl_4-induced liver injury in wistar rats. "b" represents $p < 0.01$ to control, "d" represents $p < 0.01$ to Negative control, and "f" represents $p < 0.01$ to CCL4 + Silybon (40 mg/kg).

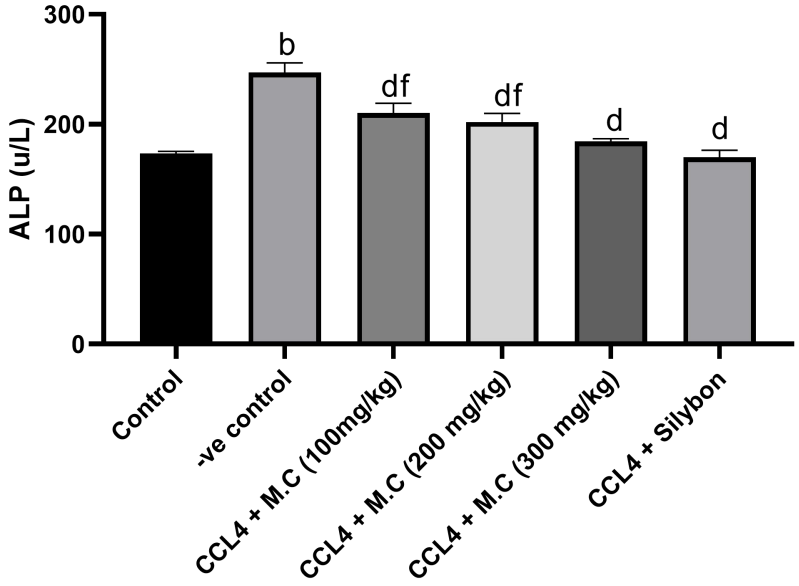


Figure 1c: The effect of *M. charantia* extract on the level of liver alkaline phosphatase in CCl_4-induced liver injury in wistar rats. "b" represents $p < 0.01$ to control, "d" represents $p < 0.01$ to Negative control, and "f" represents $p < 0.01$ to CCL4 + Silybon (40 mg/kg).

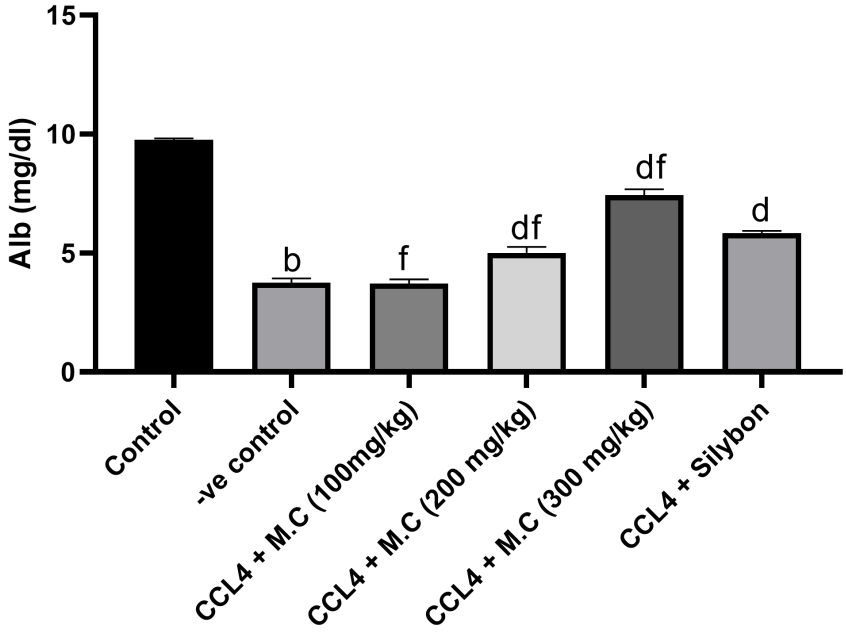


Figure 1d: The effect of *M. charantia* extract on the level of albumin in CCl_4-induced liver injury in wistar rats. b" represents $p < 0.01$ to control, "d" represents $p < 0.01$ to Negative control, and "f" represents $p < 0.01$ to CCL4 + Silybon (40 mg/kg).

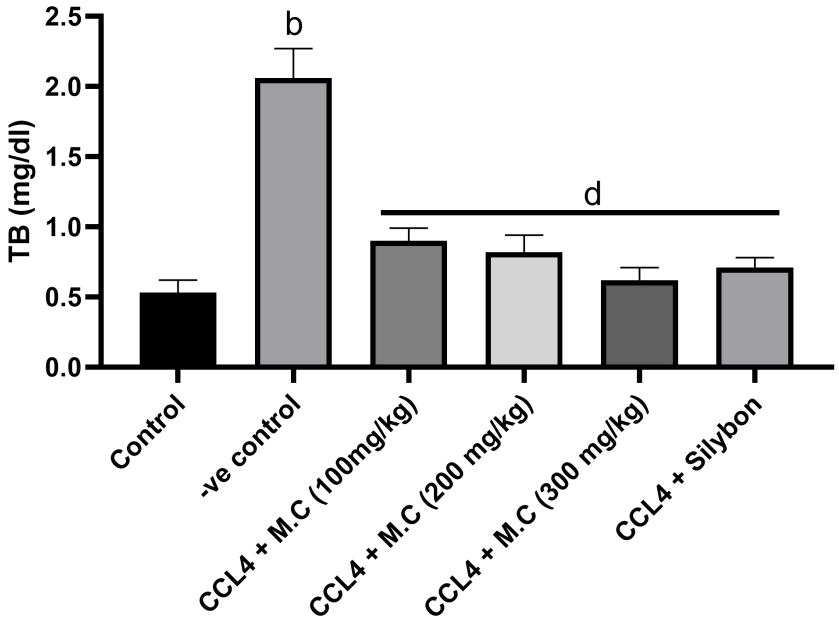
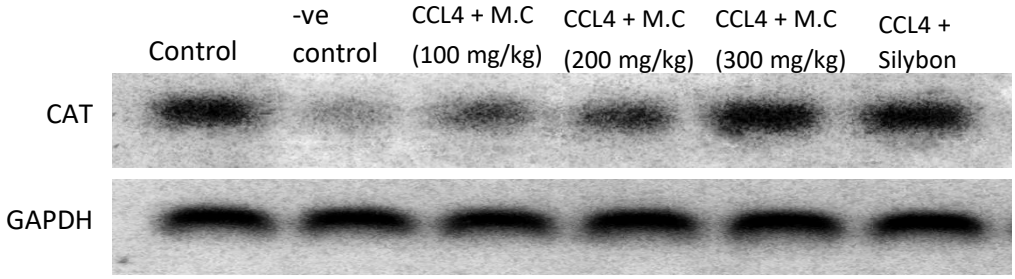


Figure 1e: The effect of *M. charantia* extract on the level of bilirubin in CCl_4-induced liver injury in wistar rats. b" represents $p < 0.01$ to control, "d" represents $p < 0.01$ to Negative control, and "f" represents $p < 0.01$ to CCL4 + Silybon (40 mg/kg).

Effect of *M. charantia* on the mRNA expression of antioxidant genes in CCl_4-induced liver injury

Figure 2a and **2b** show the effect of *M. charantia* on the expression of SOD and CAT in CCL4-induced liver injury. There was significant ($p<0.01$) downregulation of SOD and CAT mRNA expression in negative control when compared negative control. The expression of hepatic SOD and CAT mRNA expression was significantly upregulated following the treatment of the rats with vary doses of *M. charantia* (100 mg/kg, 200 mg/kg and 300 mg/kg) and silybon (40 mg/kg). However, the mRNA expression of hepatic SOD and CAT was significantly ($p<0.01$) downregulated in CCL4 + M. C (100 mg/kg) and CCL4 + M. C (200 mg/kg) when compared to CCL4 +silybon (40 mg/kg).



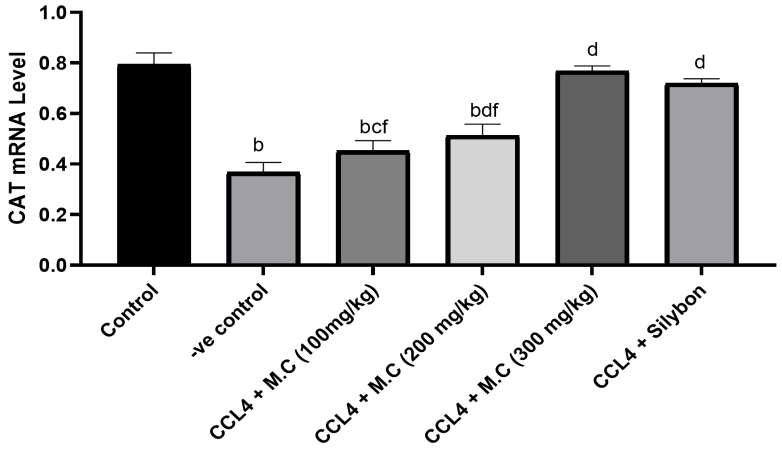
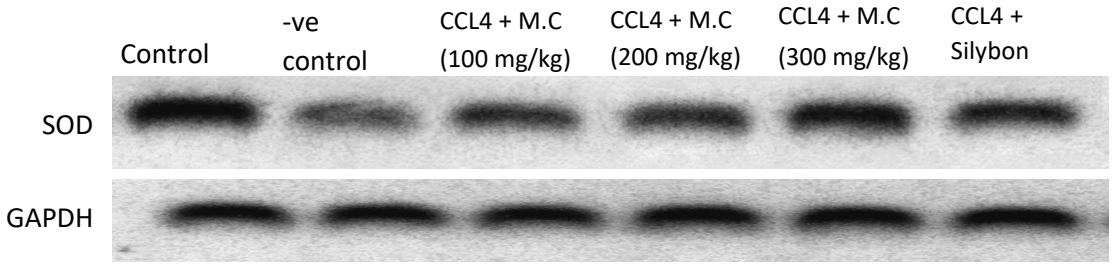


Figure 2a: Effect of *M. charantia* on mRNA expression of CAT in acute-carbon tetrachloride-induced liver injury in wistar rats. "b" represents p < 0.01 to control, "c" represents p< 0.05 to Negative control, "d" represents p < 0.01 to Negative control, and "f" represents p < 0.01 to CCL4 + Silybon (40 mg/kg).



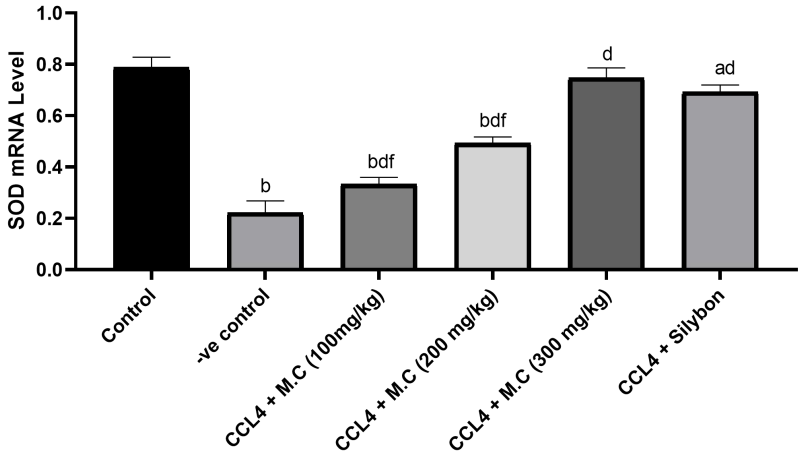


Figure 2b: Effect of *M. charantia* on mRNA expression of SOD in acute-carbon tetrachloride-induced liver injury in wistar rats. "a" represent p < 0.05 to control "b" represent p < 0.01 to control, "d" represent p < 0.01 to Negative control, "f" represent p < 0.01 to CCL4 + Silybon (40 mg/kg).

Effect of *M. charantia* on AhR-NQO1 signaling pathway of CCl_4-induced liver injury in wistar rats

The effect of *M. charantia* on hepatic mRNA expression of AhR and NQ01 in carbon tetrachloride-induced liver injury in wistar rats are shown in **Figure 3a** and **3b.** induction of liver injury significantly ($p<0.01$) downregulated the expression of AhR and NQ01 when compared to negative control. CCL4 + M. C (100 mg/kg), CCL4 + M. C (200 mg/kg), CCL4 + M. C (300 mg/kg) and CCL4 + silybon (40 mg/kg) when compared to negative control. The results also show that there is repression of AhR and NQ01 in group treated with 100 mg/kg and 200 mg/kg of *M. charantia* when compared with group treated with 40 mg/kg of silybon.

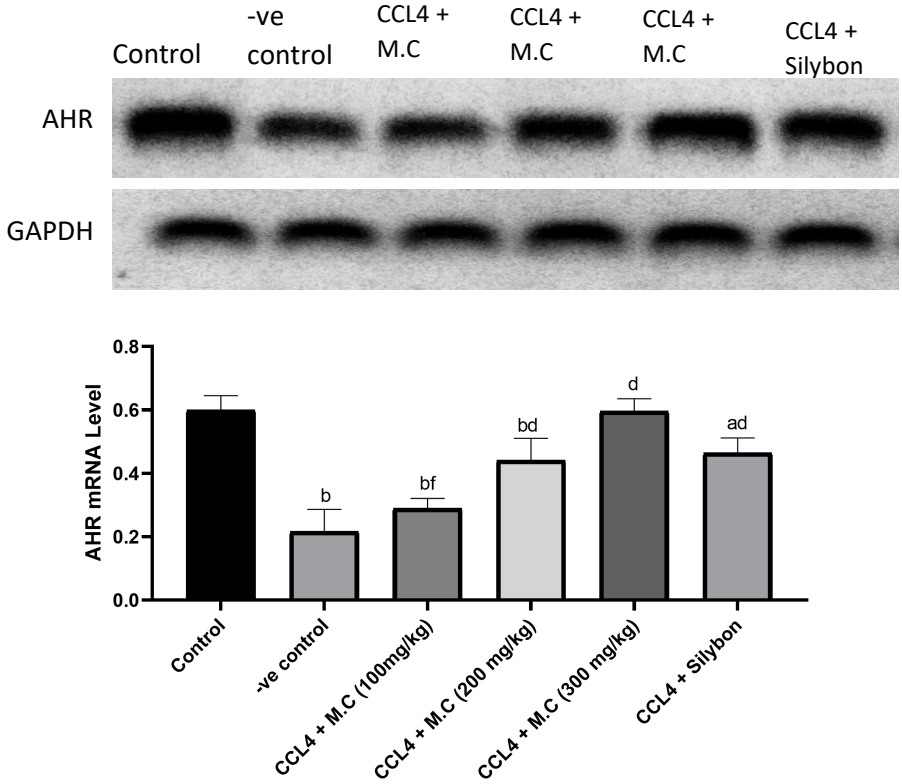
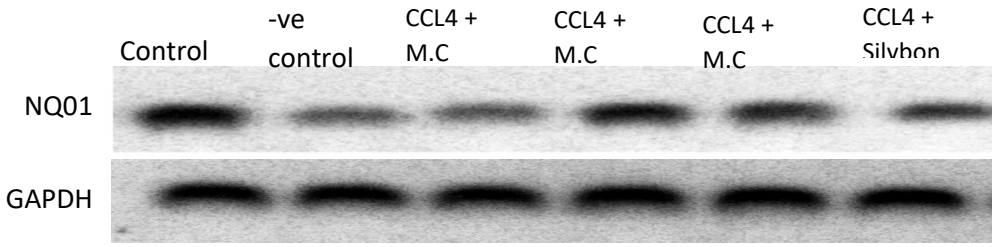


Figure 3a: Effect of *M. charantia* on mRNA expression of AHR in acute-carbon tetrachloride-induced liver injury in wistar rats. "a" represent $p < 0.05$ to control "b" represent $p < 0.01$ to control, "d" represent $p < 0.01$ to Negative control, "f" represent $p < 0.01$ to CCL4 + Silybon (40 mg/kg).



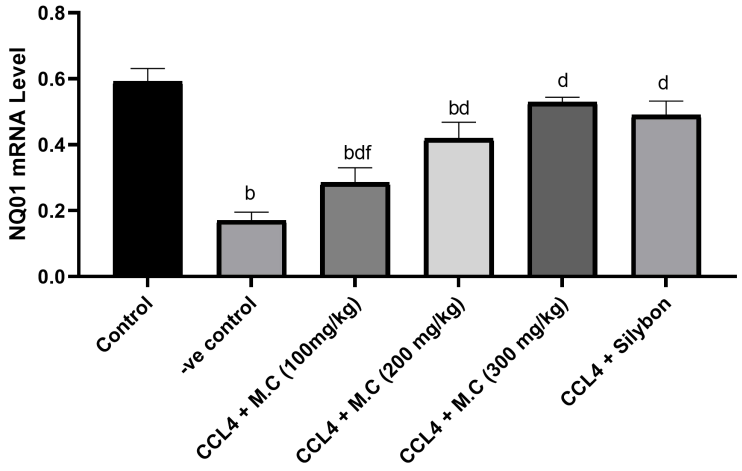
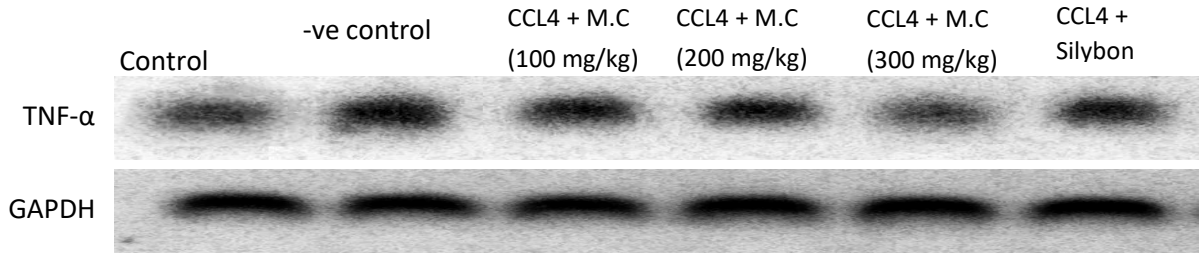


Figure 3b: Effect of *M. charantia* on mRNA expression of NQ01 in acute-carbon tetrachloride-induced liver injury in wistar rats. "b" represent $p < 0.01$ to control, "d" represent $p < 0.01$ to Negative control, "f" represent $p < 0.01$ to CCL4 + Silybon (40 mg/kg).

Effect of *M. charantia* on pro-inflammatory and anti-inflammatory cytokines in CCl_4-induced liver injury

The results (**Figure 4a, 4b, 4c** and **4d**) show that the hepatic mRNA expression of TNF-α, IL-1β, and IL-6 where significantly ($p<0.01$) overexpressed in negative control when compared with control. Oral therapy with vary doses of *M. charantia* and 40mg/kg of silybon significantly downregulated the hepatic mRNA expression of these pro-inflammatory cytokines when compared to negative control. Consequently, the results also show that the expressions of TNF-α, IL-1 β, and IL-6 were significantly upregulated in CCL4 + M. C (100 mg/kg) and CCL4 + M. C (200 mg/kg) treatment groups when compared with CCL4 + silybon (40 mg/kg).

Figure 4d shows the effect of *M.charantia* on hepatic mRNA expression on anti-inflammatory cytokines IL-10 in CCL4-induced liver damage in wistar rats. Induction of liver injury caused significant down-expression of IL-10 when compared to control. This effect was reversed following oral therapy with *M. charantia* (100 mg/kg, 200 mg/kg and 300 mg/kg) and silybon (40 mg/kg).



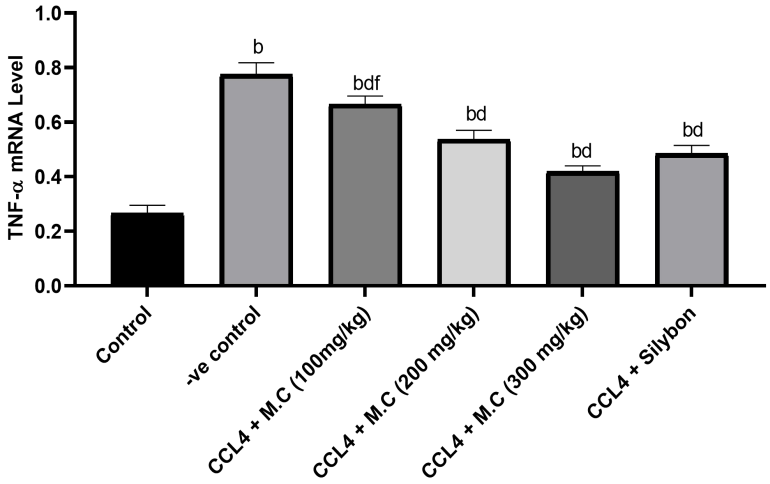
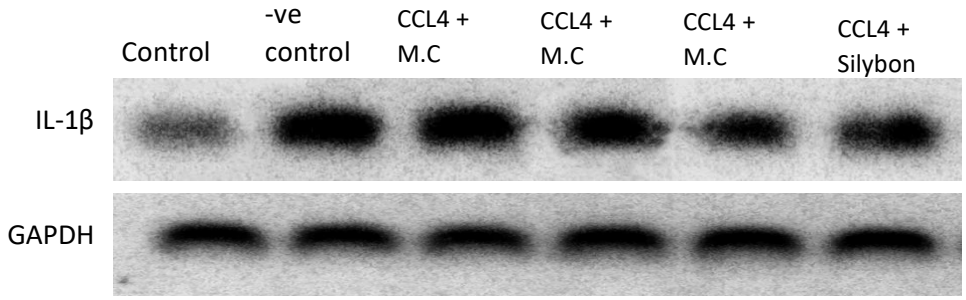


Figure 4a: Effect of *M. charantia* on mRNA expression of TNF-α in acute-carbon tetrachloride-induced liver injury in wistar rats. "b" represents p < 0.01 to control, "d" represents p < 0.01 to Negative control, and "f" represents p < 0.01 to CCL4 + Silybon (40 mg/kg).



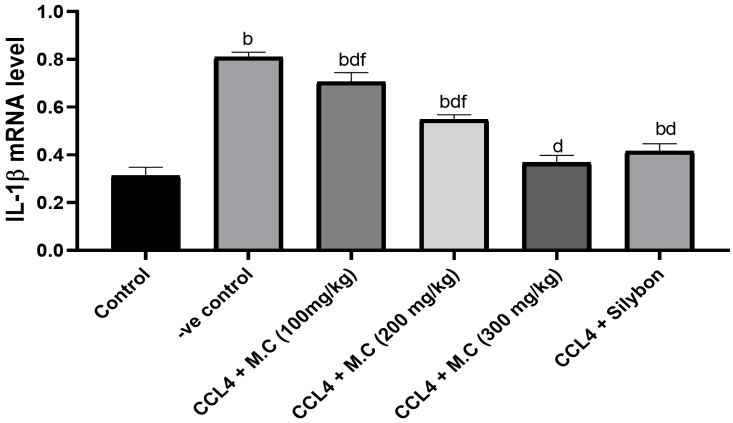
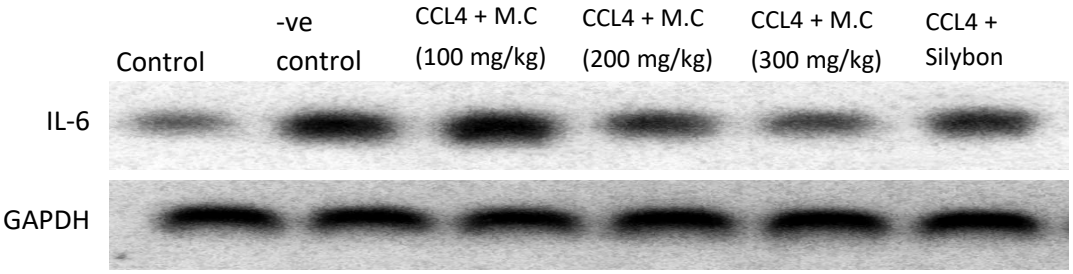


Figure 4b: Effect of *M. charantia* on mRNA expression of IL-1β in acute-carbon tetrachloride-induced liver injury in wistar rats. "b" represents p < 0.01 to control, "d" represents p < 0.01 to Negative control, "f" and represents p < 0.01 to CCL4 + Silybon (40 mg/kg).



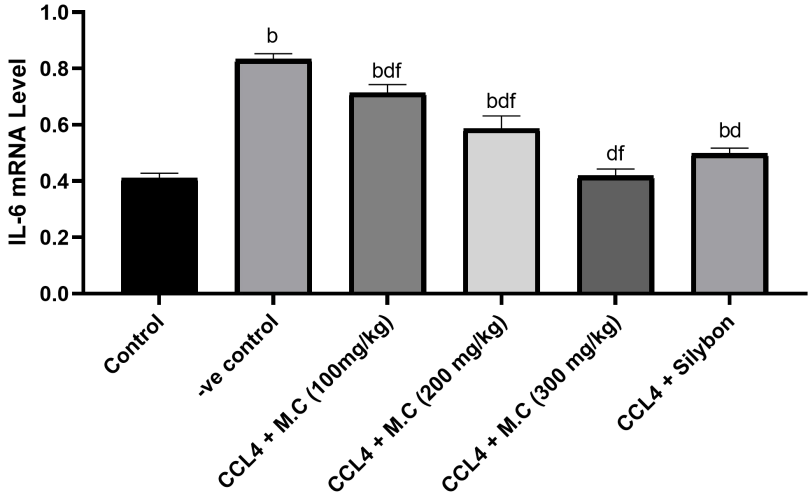
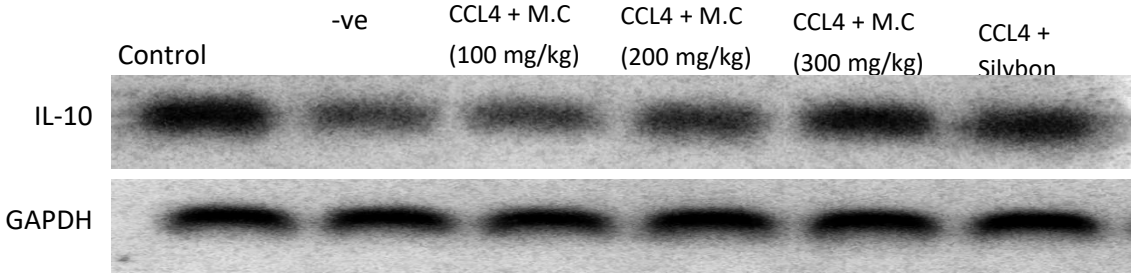


Figure 4c: Effect of *M. charantia* on mRNA expression of IL-6 in acute-carbon tetrachloride-induced liver injury in wistar rats. "b" represents p < 0.01 to control, "d" represents p < 0.01 to Negative control, "f" represents p < 0.01 to CCL4 + Silybon (40 mg/kg).



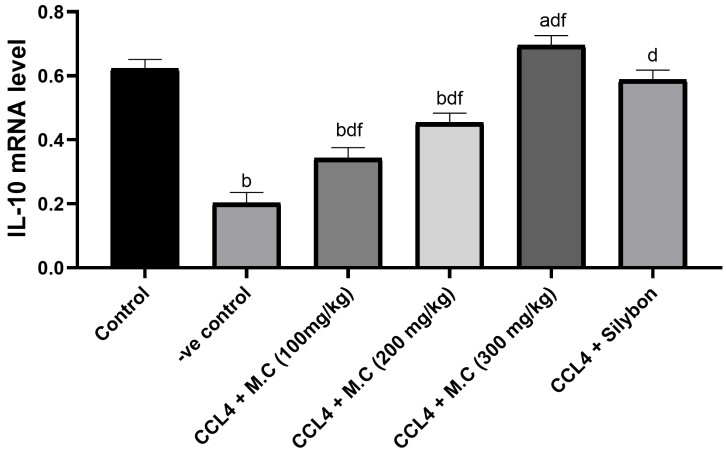


Figure 4d: Effect of *M. charantia* on mRNA expression of IL-10 in acute-carbon tetrachloride-induced liver injury in wistar rats. "a" represents $p < 0.05$ to control "b" represents $p < 0.01$ to control, "d" represents $p < 0.01$ to Negative control, "f" and represents $p < 0.01$ to CCL4 + Silybon (40 mg/kg).

Histopathological Examination

Histological study of sections from the liver of control rat, negative control rats, rats treated with varying doses of *M. charantia* and silybon (40 mg/kg) is shown in **Figure 5**. The control reveals the normal architecture of the liver with the central vein (CV), hepatocytes (H), and sinuosoid channels(S) with slight karyolysis. Microscopic examination of livers from rats administered CCl_4 (negative control) showed degeneration from the normal architectural structure with enlarged central vein (CV), blood congestion, and the sinusoid channels (S). histopathological investigation of rats treated with 100 mg/kg of *M. charantia* showed slight degeneration from the normal architectural structure of the liver with necrosis of the hepatocytes (NH). There was slight distortion of the normal Architectural Structure with sinusoid channel (S), and hepatocytes (H) in the liver of CCl_4 administered rats treated with *M. charantia*(200 mg/kg). On the other hand, microscopic investigation revealed that CCl_4 administered rats treated with 300 mg/kg of *M. charantia* had normal liver structure with intact sinusoid channel (S) and hepatocyte (H), and coupled with cellular drainage. Finally, CCl_4 administered rats treated with silybon (40mg/kg) showed normal liver structure with cellular drainage (CD) with intact sinusoid channel (S) and hepatocyte (H).

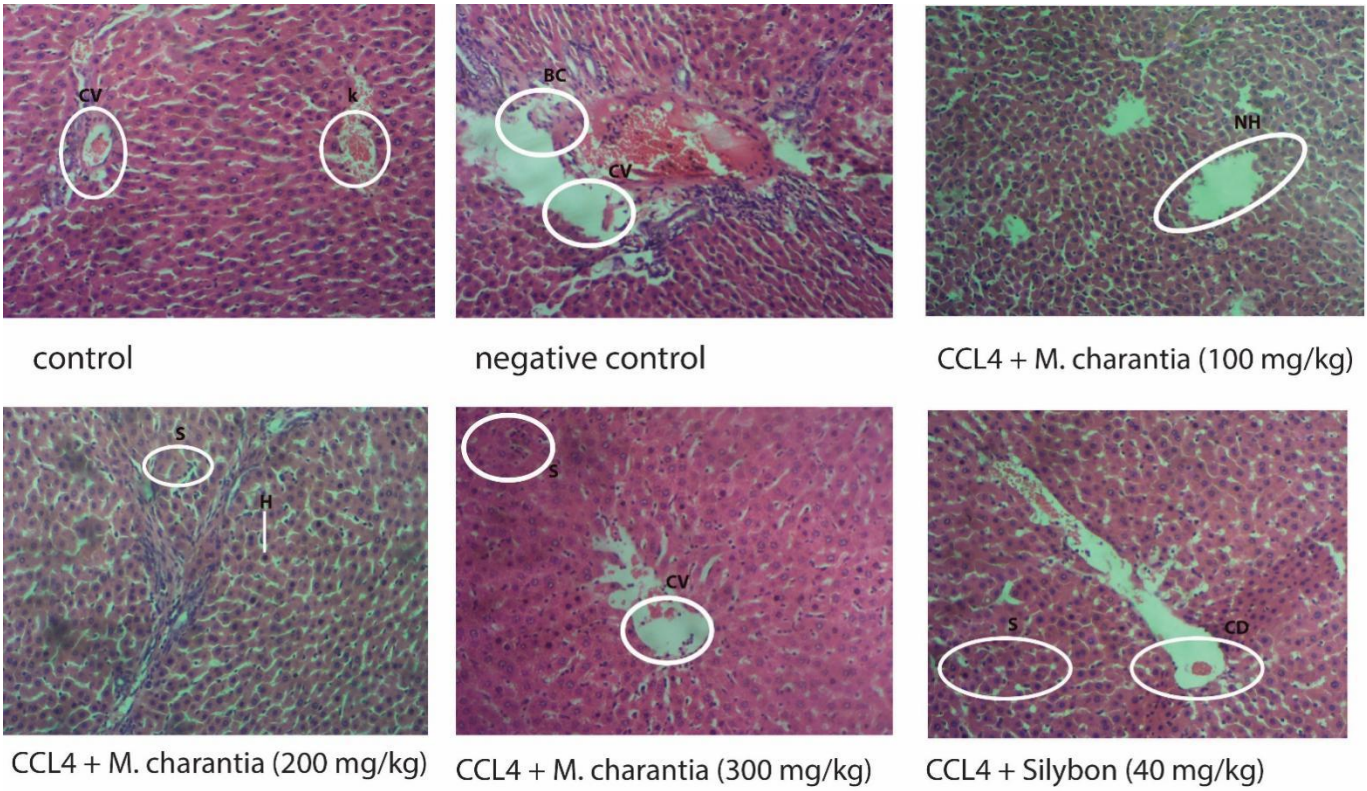


Figure 5: Photomicrograph of the liver tissues using hematoxylin and eosin (H&E) staining

Discussion

Liver plays pivotal role in the biotransformation of xenobiotics, and it is highly susceptible to damage including hepatotoxicity. Various mechanisms have been put forth to described the effect of CCl_4 in liver damage. In this study, the induction of liver injury following interperitonal injection of CCl_4 caused oxidative stress, inflammation, and histopathological damage of the liver. CCl4-treated rats developed severe inflammatory reactions, including increased levels of TNF-α, IL-6, and IL-1, as well as decreased levels of IL-10. Furthermore, lipid peroxidation increased after CCl4 treatment, which was accompanied by depleted endogenous antioxidants in the form of lower levels of CAT, SOD, AHR, and NQ01. As a result of CCl4-induced liver damage, hepatocytes lost their membrane integrity and ability to conjugate or excrete bilirubin, as evidenced by increases in serum bilirubin and liver enzyme levels activities (ALT, AST, ALP, and Alb), which is consistent with previous findings of [27,28].

When compared to the control group, the administration of CCL4 to rats resulted in a significant increase in the number of white blood cell, as well as a significant decrease in the number of red blood cells along with Hb, PCV and platelets parameters. Plasma membranes of blood cellular elements have been reported to be sensitive to oxidative stress. This sensitivity has been attributed to the high percentage of polyunsaturated fatty acids that they contain [29]. RBC count and other haematological parameters may decrease as a result of inducing free radicals such as trichloromethyl proxy free radical (CCl3OO) and trichloromethyl free radical (CCl3). This is because inducing free radicals can lead to the formation of free radicals [30]. In addition, *Hussien* et al. [31]

showed that erythrocyte membrane damage from free radicals contributes to the release of haemoglobin from cells and a reduction in hemoglobin content. As previously reported by *Elshater et al* [32], who stated the ability of free radicals to disrupt the WBCs count of CCL4 treated rats, the increase in WBCs count in CCL4 treated rats observed in this study could be considered a defensive immune system mechanism [25]. The results of the current study showed that, in comparison to the CCL4-treated group, hematological blood parameters were improved by treatment with 100 mg/kg, 200 mg/kg, and 300 mg/kg *M*. *charantia* and 40 mg/kg silybon. This finding was supported by a significant ($P< 0.05$) rise in RBC, Hb, PCV and PLT levels as well as a fall in WBC levels. (Table 2)

Our findings are in line with earlier studies by *Moqbel et al* [33] that demonstrated that *M*. *chaantia* reduces streptozotocin-induced PCV, Hb, and WBC counts disturbances; Ranasinghe *et al* [34] showed an increase in *M*. *charantia's* effects on RBC count in rat model of hepatocellular carcinoma; and Divya *et al* [35] demonstrated the reversal effect of *M*. *charantia* on hemoglobin concentration, white blood cell count, and platelet count, establishing it as a potential new source of antioxidants and natural immune modulators.

It has been established that oxidative stress and inflammatory responses are key pathophysiologic mechanisms for liver damage [36]. Production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are crucial for preserving the body's homeostasis and serves as a crucial regulatory mediator for cell-cell communication or the activation of specific signalling pathways. However, when there is an excess of ROS/RNS in the cellular system, they interact with DNA, lipid, and protein molecules and harm the cellular system. Under normal circumstances, harmful ROS/RNS react with antioxidant systems like catalase (CAT) and superoxide dismutase (SOD), converting them into less toxic molecules. When ROS/RNS overproduction exceeds the antioxidant defense system or antioxidants that are necessary to neutralize oxidants are ineffective, pathological conditions give rise to the stress conditions known as oxidative stress [37,38].

NrF_2 is a significant regulator of the antioxidant response. It regulates a number of antioxidant enzymes, including NQO1 and HO-1, by binding to its proper binding sites, which are also known as antioxidant response elements after nuclear translocation. Another AhR target gene is NQO1, which has AhR binding sites known as xenobiotic response element binding sites [39]. In this study, we were able to show that rats treated with CCl4 had lower levels of AhR and NQ01 mRNA expression. Previous studies showed that NQO1 expression is dependent on AhR because AhR deletion decreased NQO1 expression in liver injury [36,40]. In this study, M. *charantia* administration raised the levels of NQ01 and AhR. According to reports, M. *charantia's* antioxidant properties are due to the presence of chemical compounds including flavonoids, saponins, ascorbic acid, phenols, beta carotene, caffeic acid, catechin, triterpenoids, tannins, gallic acid, and other phenolic compounds [41,42]. These compounds have the ability to scavenge free radicals, prevent lipid peroxidation, and inhibit xanthine oxidase, which could make free radicals into more stable products and eventually stop free radical chain reactions [43].

Non-alcoholic fatty liver disease, severe fibrinogenesis, and hepatocellular carcinoma all have inflammation as a common factor because it is thought to be one of the primary causes of liver tissue damage. Pro-inflammatory cytokines like IL-1β, IL-6, and TNF-α have been found to be released in response to CCl4-induced liver toxicity, which has been linked to inflammation [44]. Hepatic injury is associated with elevated levels of the inflammatory cytokines IL-1β and TNF-α [45]. TNF-α is a pro-inflammatory cytokine that plays a crucial role in the etiology and progression of many different types of acute and chronic liver disease. Carbon tetrachloride induced liver damage included an inflammatory phase in which TNF-α exacerbated damage from oxidative stress and inflammation [46]. Hepatocytes, stellate cells, and endothelial cells can all be activated by TNF-α; these cells, in turn, release chemokines that attract and activate

circulating inflammatory cells, thereby further stimulating inflammation. In addition, TNF-α causes necrosis by releasing elastases and inducing ROS production by Kupffer cells. The activation and hyperplasia of Kupffer cells serve both a protective and potentially harmful function [47]. The production of hepatoprotective cytokines (IL-10) in the process of hepatocyte regeneration indicate the protective role. On the other hand, the production of pro-inflammatory cytokines indicates the harmful role in the development of chronic inflammatory diseases of the liver, such as alcoholic liver disease (ALD), non-alcoholic steatohepatitis (NASH), and non-alcoholic fatty liver disease (NAFLD) [48,49]. In addition to contributing significantly to lipid peroxidation, the trichloromethyl radical plays a crucial role in inducing inflammation by stimulating Kupffer cells in the liver. Kupffer cell activation leads to the production and release of inflammatory mediators (TNF-α, IL-6, and IL-1β), which further contribute to the destruction of the parenchymal liver cells [50]. In the CCl4-treated rats, our study showed that the expression of the pro-inflammatory genes TNF- α, IL-6, and IL-1β increased while the expression of the anti-inflammatory gene IL-10 significantly decreased. Interestingly, interactions between AhR and NF-kB pathways may be crucial for regulating inflammatory reactions and diseases associated with them [51]. According to previous research Huang X, *et al* and Li [13,52], AhR whole-body knockouts exhibit elevated levels of TNF- α, IL-6, and IL-1 β in an experimental model of autoimmune uveitis, and peritoneal macrophages isolated from AhR null mice also exhibit elevated levels of these cytokines [53]. M. *charantia's* active components can increase the expression of the anti-inflammatory gene IL-10 while decreasing the expression of TNF- α and other proinflammatory genes, such as IL-6 and IL-1 β [43].

The central vein and hepatocytes in the control rats were seen to be organized into cords or plates and to be separated by hepatic sinusoids in the histological observations of the hepatoprotective effects of *M. charantia* on CCl4-induced liver damage in this study. Administration of CCl4 resulted in a number of changes in the histological structure of the liver, including an alteration in the hepatic lobules' general morphology. Around the central veins and between hepatocytes, there are numerous areas where collagen fibers have been deposited. Administration of 100 mg/kg and 200 mg/kg *M. charantia* extract along with CCl4 showed slight improvement in liver histology, On the other hand, recovery in most areas, hepatocytes well preserved and no area of necrosis was observed in the 300 mg/kg *M. charantia* and Silybon treated rats. CCl4 caused severe histological changes in liver tissue, necrosis, inflammation, congestion, and sinusoidal dilatation, as shown by the histological findings of this study, which confirmed the haematological and biochemical findings Comparable histological changes in the CCl4-induced liver injury have been recorded previously; [54] that CCL4 initially induced cell death of a pericentral ring of hepatocytes followed by destruction of the characteristic microarchitecture of the hepatic lobules. Another study of Li *et al* [55] revealed by histopathological results, the CCl4-induced chronic and acute liver injuries; Also, necrotic changes in the centrilobular area with swelling of the hepatocytes and infiltration of ceroid pigment-laden macrophages was observed by Geerts *et al* [56] indicating fibrotic changes in the centrilobular area. It could be observed that treatment with *M. charantia* used in this study protected rats effectively against CCl4. It reduced necrosis and other histopathological changes caused by CCl4 toxicity.

CONCLUSION

In conclusion, administration of *M. charantia* demonstrated significant hepatoprotection against CCl4 induced liver injury in wistar rats. *M. charantia* significantly reduced the inflammatory and oxidative stress markers while improving the hematological, biochemical, and histological parameters. Additionally, there was no detectable toxicity against the animals from *M. charantia* treatment. Therefore, *M. charantia* can demonstrate potential therapeutic effect against the liver injury based on the current study.

Authors' contribution Statement

All authors have accepted responsibility for the entire content of this manuscript and approved its

submission. HBO: conceptualization, project design and supervision; NEB: data analysis,

methodology, original drafting of the manuscript; OOE: d project design and supervision; O.F.K:

manuscript editing; O.I: methodology and data analysis

Data Availability

The data used for this study has been provided in the manuscript

Ethical Declaration

The experiments were performed in accordance with the National Guidelines for Experimental

Animal Welfare and with approval of the Animal Welfare and Research Ethics Committee at

Federal university of Technology, Akure, Nigeria (Ethical approval No: FUTA/ETH/2020/013).

Declaration of competing interest

The authors declare no conflict of interest

Acknowledgments

Not applicable.

Research funding

The expenditure of research we done by the researcher.

References

1. Trefts E, Gannon M, Wasserman DH. The liver. Curr Biol. 2017;27(21):1147-1151.
2. Ma Q. Transcriptional responses to oxidative stress: Pathological and toxicological implications. Pharmacol. Ther. 2010;125:376–393.
3. Asrani, S.K.; Devarbhavi, H.; Eaton, J.; Kamath, P.S. Burden of liver diseases in the world. Journal of Hepatology. 2019;70:151–171.
4. Ammar NM, Hassan HA, Abdallah HMI, Afifi SM, Elgamal AM, Farrag ARH, El-Gendy AE-NG, Farag MA, Elshamy AI. Protective Effects of Naringenin from Citrus sinensis (var. Valencia) Peels against CCl_4-Induced Hepatic and Renal Injuries in Rats Assessed by Metabolomics, Histological and Biochemical Analyses. Nutrients. 2022;14(4):841.
5. Hernández-Aquino, E.; Muriel, P. Benefcial effects of naringenin in liver diseases: Molecular mechanisms. World J. Gastroenterol. 2018;24:1679–1707.
6. Mesalam, N. M., Aldhumri, S. A., Gabr, S. A., Ibrahim, M. A., Al-Mokaddem, A. K., and Abdel-Moneim, A. M. E. (2021). Putative abrogation impacts of Ajwa seeds on oxidative damage, liver dysfunction and associated complications in rats exposed to carbon tetrachloride. *Molecular Biology Reports*, *48*(**6**): 5305-5318
7. Makni, M., Chtourou, Y., Garoui, E. M., Boudawara, T., and Fetoui, H. Carbon tetrachloride-induced nephrotoxicity and DNA damage in rats: protective role of vanillin. Human and Experimental Toxicology. 2012;31:844–852
8. Deng, J.S.; Chang, Y.C.; Wenc, C.L.; Liao, J.C.; Hou, W.C.; Amagaya, S.; Huang, S.S.; Huang, G.J. Hepatoprotective effect of the ethanol extract of Vitisthun bergii on carbon tetrachloride-induced acute hepatotoxicity in rats through anti-oxidative activities. J. Ethnopharmacol. 2012, 142, 795–803.
9. Li, Z., Liu, X., Zhang, P., Han, R., Sun, G., Jiang, R., Wang, Y., Liu, X., Li, W., Kang, X and Tian, Y. Comparative transcriptome analysis of hypothalamus-regulated feed intake induced by exogenous visfatin in chicks. BMC Genomics. 2018;19(**1**):249.

10. Sagor A T , Mohammed R. H C., Tabassum N, Hemayet H , Rahman M. M and Alam M. A: Supplementation of fresh ucche (Momordica charantia L. var. muricata Willd) prevented oxidative stress, fibrosis and hepatic damage in CCl4 treated rats. BMC Complementary and Alternative Medicine 2015;15:115 DOI 10.1186/s12906-015-0636-1
11. Huabing Tan, Qin He, Rugui Li, Feifei Lei & Xu Lei. Trillin Reduces Liver Chronic Inflammation and Fibrosis in Carbon Tetrachloride (CCl4) Induced Liver Injury in Mice, Immunological Investigations, 2016;45(5):371-382,
12. Unsal V, Cicek M, Sabancilar İ. Toxicity of carbon tetrachloride, free radicals and role of antioxidants. Rev Environ Health. 2020;36(2):279-295.
13. Huang X, et al. Extract of AverrhoacarambolaL.(Oxalidaceae) roots ameliorates carbon tetrachloride-induced hepatic fibrosis in rats. Biomed Pharmacother2020;121:109516.
14. Kolios G, Valatas V, Kouroumalis E. Role of Kupffer cells in the pathogenesis of liver disease. World J Gastroenterol. 2006;12(46):7413-20.
15. Ullah, H., Khan, A., Baig, M.W., Ullah, N., Ahmed, N., Tipu, M.K., Ali, H. and Khan, S., 2020. Poncirin attenuates CCL4-induced liver injury through inhibition of oxidative stress and inflammatory cytokines in mice. BMC complementary medicine and therapies, 20(1), pp.1-14.
16. Sahreen S, Khan MR, Khan R. A Ameliorating effect of various fractions of Rumex hastatus roots against hepato- and testicular toxicity caused by CCl4. Oxid Med Cell Longev2013;2013:325406.
17. Giordano C, Rivas J, Zervos X. An Update on Treatment of Drug-Induced Liver Injury. J Clin Transl Hepatol. 2014;2(2):74-9.
18. Jonathan G. Stine & James H. Lewis. Current and future directions in the treatment and prevention of drug-induced liver injury: a systematic review, Expert Review of Gastroenterology and Hepatology, 2016;10(4): 517-536,
19a. Kren V, Walterova D. Silybin and Silymarin- New effects and applications. Biomed Papers. 2005; 149: 29–41
19. Triantafyllidi A, Xanthos T, Papalois A, J. Pharm. Sci. Rev. Res Triantafillidis JK. Herbal and plant therapy in patients with inflammatory bowel disease. Ann Gastroenterol. 2015;28(**2**):210-220.
20. Gupta, M., Sharma, S., Gautam, A. K., and Bhadauria, R.. Momordica charantia Linn, (karela): nature's silent healer. Int.. 2011;**11**, 32–37.
21. Elekofehinti, O.O., Oyedokun, V.O., Iwaloye, O. et al. Momordica charantia silver nanoparticles modulate S**OCS**/JAK/STAT and P13K/Akt/PTEN signalling pathways in the kidney of streptozotocin-induced diabetic rats. J Diabetes Metab Disord . 2021;**20,** 245–260.
22. El-Haskoury, R., Al-Waili, N., Kamoun, Z., Makni, M., Al-Waili, H., & Lyoussi, B. (2018). Antioxidant activity and protective effect of carob honey in CCl4-induced kidney and liver injury. Archives of medical research, 49(5), 306–313
23. Bradford, N. A rapid and sensitive method for the quantitation microgram quantities of a protein isolated from red cell membranes. Anal. Biochem, 1976;72:248, e254.
24. Reitman, S., & Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology, 1957;28(1), 56–63.
25. Englehardt, A. Measurement of alkaline phosphatase. Aerztl Labor, 1970;16(42), 1.
26. Graham, M. D. (2022). The Coulter Principle: A history. Cytometry Part A. Wiley Online Library.
27. Mazin A. Zamzami, Othman A. S. Baothman, Fatma Samy, Mohamed Kamel Abo-Golayel, "Amelioration of

CCl_4-Induced Hepatotoxicity in Rabbits by Lepidium sativum Seeds", Evidence-Based Complementary and Alternative Medicine, vol. 2019, Article ID 5947234, 17 pages, 2019.

28. Alqasoumi S. I. and M. S. Abdel-Kader, "Screening of some traditionally used plants for their hepatoprotective effect," in Phytochemicals as Nutraceuticals - Global Approaches to Their Role in Nutrition and Health, 2011.
29. Noha, M. M., Suzan, A. I., Nagy, S. T. Protective effects of honey and bee venom against lipopolysaccharide and carbon tetrachloride-induced hepatoxicity and lipid peroxidation in rats, Toxicology Research, 2020;9(5): 693-705.
30. Ibrahim, Z. S., Nassan, M. A., Soliman, M. M. Ameliorative effects of pomegranate on carbon tetrachloride hepatotoxicity in rats: a molecular and histopathological study. Mol Med Rep. 2016;13: 3653–60.
31. Hussien, E. M., Darwish, M. M., Ali, S. E. Prophylactic role of combined treatment with coenzyme Q 10 and vitamin E against radiation in male rats. Egypt J Rad Sci Appl. 2007;20: 181–94.
32. Elshater, A. A., Salman, M. M., Mohamed, S. A. The hepatoameliorating effect of Solanum nigrum against CCL_4 induced liver toxicity in albino rats. Egypt Acad J Biol Sci. 2013;5: 59–66.
33. Moqbel, M.S., Al- Hizab, F.A., Barakat, S.M. Clinicopathological Study on the Effects of Momordica charantia on Streptozotocin-Induced Diabetic Wistar Rats. Open Journal of Veterinary Medicine, 2017;7, 49-62.
34. Ranasinghe, K. N. K., Premarathna, A. D., Mahakapuge, T. A. N., Wijesundera, K. K., Ambagaspitiya, A. T., Jayasooriya, A. P., Kularatne, S. A. M., Rajapakse, R. P. V. J. In vivo anticancer effects of Momordica charantia seed fat on hepatocellular carcinoma in a rat model, Journal of Ayurveda and Integrative Medicine, 2021;12(3): 435-442,
35. Divya, D., Hettiarachchy, N. S., Ganesh, V., Kanna, A., Rayaprolu, S. Phenolic extract from leaves of Momordica charantia with antioxidant properties. J Agric Sci Appl 2013;2 (1):28- 34.
36. Ambade A. , Mandrekar. P. Oxidative stress and inflammation: essential partners in alcoholic liver disease. Int J Hepatol, 2012; p. 853175
37. Ma, Q. Role of nrf2 in oxidative stress and toxicity. Annu. Rev. Pharmacol. Toxicol. 2013;53: 401–426.
38. Ma, Y., Lee, G., Heo, S. Y., and Roh, Y. S. Oxidative stress is a key modulator in the development of nonalcoholic fatty liver disease. Antioxidants, 2021;11(**1**): 91.
39. Dietrich C. Antioxidant functions of the aryl hydrocarbon receptor. Stem Cells Int, 2016; p. 7943495
40. Haibo Dong, Liuyi Hao, Wenliang Zhang, Wei Zhong, Wei Guo, Ruichao Yue, Xinguo Sun, Zhanxiang Zhou. Activation of AhR-NQO1 Signaling Pathway Protects Against Alcohol-Induced Liver Injury by Improving Redox Balance. Cellular and Molecular Gastroenterology and Hepatology, 2021;12(3):793-811,
41. DeFeudis F V, Papadopoulos V, Drieu K. Ginkgo biloba extracts and cancer: a research area in its infancy. Fundam Clin Pharmacol. 2003;17(4):405–17.
42. Gary R. Takeoka, Dao LT. Antioxidant Constituents of Almond [Prunus dulcis (Mill.) D.A. Webb] Hulls. 2002; Available from: https://pubs.acs.org/doi/abs/10.1021/jf020660i
43. Li, Z., Xia, A., Li, S., Yang, G., Jin, W., Zhang, M., & Wang, S. (2020). The Pharmacological Properties and Therapeutic Use of Bitter Melon (Momordica charantia L.). Current Pharmacology Reports. doi:10.1007/s40495-020-00219-4
44. Naji, K.M.; Al-Shaibani, E.S.; Alhadi, F.A.; Al-Soudi, S.A.; D'Souza, M.R. Hepatoprotective and antioxidant effects of single clove garlic against CCl4-

induced hepatic damage in rabbits. BMC Complement. Altern. Med. 2017;17:1–12.
45. Tsai, J.-C.; Chen, Y.-A.; Wu, J.-T.; Cheng, K.-C.; Lai, P.-S.; Liu, K.-F.; Lin, Y.-K.; Huang, Y.-T.; Hsieh, C.-W. Extracts from Fermented Black Garlic Exhibit a Hepatoprotective Effect on Acute Hepatic Injury. Molecules. 2019;24:1112.
46. Li, X., Wang, L., and Chen, C. Effects of exogenous thymosin β4 on carbon tetrachloride-induced liver injury and fibrosis. Scientific Report. 2017;7:5872
47. Bami, E.; Ozakpınar, O.B.; Ozdemir-Kumral, Z.N.; Köroglu, K.; Ercan, F.; Cirakli, Z.; Sekerler, T.; Izzettin, F.V.; Sancar, M.; Okuyan, B. Protective effect of ferulic acid on cisplatin induced nephrotoxicity in rats. Environ. Toxicol. Pharmacol. 2017;54:105–111.
48. Bocsan, I.C.; Milaciu, M.V.; Pop, R.M.; Vesa, S.C.; Ciumarnean, L.; Matei, D.M.; Buzoianu, A.D. Cytokines Genotype-Phenotype Correlation in Nonalcoholic Steatohepatitis. Oxid. Med. Cell Longev. 2017;4297206.
49. Dixon, L.J.; Barnes, M.; Tang, H.; Pritchard, M.T.; Nagy, L.E. Kupffer Cells in the Liver. Compr. P hysiol. 2013;3:785–797.
50. Popović, D.; Kocić, G.; Katić, V.; Zarubica, A.; Janković Veličković, L.; Ničković, V.P.; Jović, A.; Veljković, A.; Petrović, V.; Rakić, V.; Jović, Z.; Poklar Ulrih, N.; Sokolović, D.; Stojanović, M.; Stanković, M.; Radenković, G.; Nikolić, G.R.; Lukač, A.; Milosavljević, A.; Sokolović, D. Anthocyanins Protect Hepatocytes against CCl_4-Induced Acute Liver Injury in Rats by Inhibiting Pro-inflammatory mediators, Polyamine Catabolism, Lipocalin-2, and Excessive Proliferation of Kupffer Cells. Antioxidants 2019;8:451.
51. Li J., Phadnis-Moghe, A.S., Crawford, R.B., Kaminski, N.E.,. Aryl hydrocarbon receptor activation by 2,3,7,8-tetrachlorodibenzo-p-dioxin impairs human B lymphopoiesis. Toxicology. 2017;378:17–24.
52. Climaco-Arvizu, S., Dominguez-Acosta, O., Cabanas-Cortes, M.A., Rodriguez-Sosa, M., Gonzalez, F.J., Vega, L., Elizondo, G.,. Aryl hydrocarbon receptor influences nitric oxide and arginine production and alters M1/M2 macrophage polarization. Life Sci. 2016;155:76–84.
53. Chunyan Wang, Michael C. Petriello, Beibei Zhu, Bernhard Hennig, PCB 126 induces monocyte/macrophage polarization and inflammation through AhR and NF-κB pathways, Toxicology and Applied Pharmacology, 2019;367;71-81.
54. Yamamoto, K., Ishii, M., Inoue, Y., et al.,. Prediction of postoperative liver regeneration from clinical information using a data-led mathematical model. Scientific Reports. 2016;6(10):1038.
55. Li, X., Zhang, F., Wang, D., Li, Z., Qin, X., and Du, G. NMR-based metabonomic and quantitative real-time PCR in the profiling of metabolic changes in carbon tetrachloride induced rat liver injury. Journal of Pharmacuetical Biomedical Analysis. 2014;15(89):42-49.
56. Geerts, A., Vanheule, E., Praet, M., Van Vlierberghe, H., De Vos, M., Colle, I. Comparison of three research models of portal hypertension in mice: macroscopic, histological and portal pressure evaluation. Int J Exp Path., 2008;89:251–263.