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Protective Potential of Momordica. charantia against CCl4 Induced Change in Liver and Kidney Serum Biomarkers in Wistar Rats

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Abstract

Mormodica charantia anti-inflammatory and antioxidant properties are regarded as crucial factors in reducing liver damage and fibrosis. Fibrosis, inflammation, and apoptosis are the hallmarks of acute liver injury, which can result in liver failure, cirrhosis, or cancer and have an impact on the clinical outcome over the longterm. The medicinal value of *M. charania* is derived from its bioactive phytochemical components, which have observable physiological effects on the body and act as a preventive measure. The aim of this present study was to investigate the effects of *M. charantia* on acute liver and kidney injury induced by carbon tetrachloride (CCL_4) in wistar rats. Wistar rats were subjected to intraperitoneal injection of 1 ml/kg body weight CCL_4 with or without *M. charantia* (100 mg/kg, 200 mg/kg and 300 mg/kg). Rats treated with CCL_4 developed acute liver and kidney injury, as evidenced by elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), urea, creatinine and accompanied with histological analysis. The present study highlighted that *M. charantia* exhibited liver and kidney protective effects against acute hepatic injury induced by CCL_4 via suppressing inflammation and oxidative stress. Administration of M. charantia demonstrated significant hepatoprotection and renal protection against CCl4 induced liver and kidney injury in wistar rats

Key words: *Mormodica charantia*, liver injury, kidney 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 (Trefts *et al*., 2017; Ma *et al*., 2019). 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 (Trefts *et al*., 2017). Liver diseases are responsible for the deaths of around 2 million people per year all over the world (Asrani *et al*., 2019; Ammar *et al*., 2022). Excessive consumption of alcohol, viral infection, blockage of the biliary system and exposure to heavy metals are all risk factors of liver damage (Hernández-Aquino and Muriel, 2008) In humans, kidney which makes up less than 1 percent of total body mass, play a vital role in the body by filtering and concentrating various substances and chemical agents, and these agents may reach hazardous level of concentration in the kidney (Schnaper, 2014).

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 Ccl link, forming a radical anion that breaks down into trichloromethyl (CCl3) and chloride (Makni *et al*., 2012; Deng, 2018). 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 peroxy radicals. 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 (Makni *et al*., 2012; Sagor *et al*., 2015; Li *et al*., 2018).

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 (Huabing *et al*., 2016; Unsal *et al*., 2020). 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. The liver and kidney serum marker enzyme concentrations are markedly elevated after CCl4 administration. It increases the levels of serum enzymes like Alanine Transaminase (ALT), Aspartate Transaminase (AST), serum bilirubin, urea and creatinine that are typically present in the cytoplasm (Sahreen *et al*., 2013; Huang *et al*., 2020).

Despite significant advances in medicine, there is no effective treatment that stimulates liver and kidney functions, protects the liver and kidney from injury and aids in the regeneration of hepatic cells (Giordano *et al*., 2014; Jonathan *et al*., 2016). As an alternative or supplement to conventional treatment, herbal medicines have garnered significant attention, and the need for these cures is now on the rise (Triantafyllidi *et al*., 2015). Studies have shown that *M. charantia* has diverse pharmacological activities including wound healing properties owing to its powerful antioxidant properties (Gupter *et al*., 2011; Elekekofehinti *et al*., 2021). Despite the enriched pharmacological properties of *M. charantia*, there is a dearth of information on the protective function in liver injury. Hence, this study investigates the protective effect of *M. charantia* on CCL_4-induced liver and kidney injury in wistar rats.

Aim of the Study:
The aim of the study was to investigate the remediative effects of *M. charania* on acute liver and kidney injury induced by carbon tetrachloride (CCL_4) in wistar rats by assessment of some liver and kidney biomarkers.

Materials and methods

Collection of plant material
The leaves of *M. charantia* was cultivated within the University of Benin Main Campus, the plant material was taken to the 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) were weighed using a weighing balance and was added to 1590 ml of methanol. The mixtures were allowed to stand for 48 hours, 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 a methanol extract of *M. charantia.* The extract was stored at room temperature (25^{o}C).

Animal purchase and acclimatization
Fifty-six (56) male albino rats 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

Estimation of liver enzyme assays
The activities of aspartate aminotransferase (AST) and the level of total protein was determined based on Bradford *et al.* (1976) using a commercially available kit (Agappe, Switzerland). The level of alanine aminotransferase (ALT) level was determined based on the method of Reitman and Frankel (1957) using a commercially available kit (Randox laboratories UK). Alkaline Phosphatase (ALP) level was determined based on the method of Englehardt *et al.* (1970) using the commercially available kit (Randox laboratories)

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

Figure 1: 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 CCl4-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 a 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 ($p<0.05$) in the level of AST and ALP following treatment with 100mg/kg and 200 mg/kg of *M. charantia* when compared with CCl4 + Silybon group

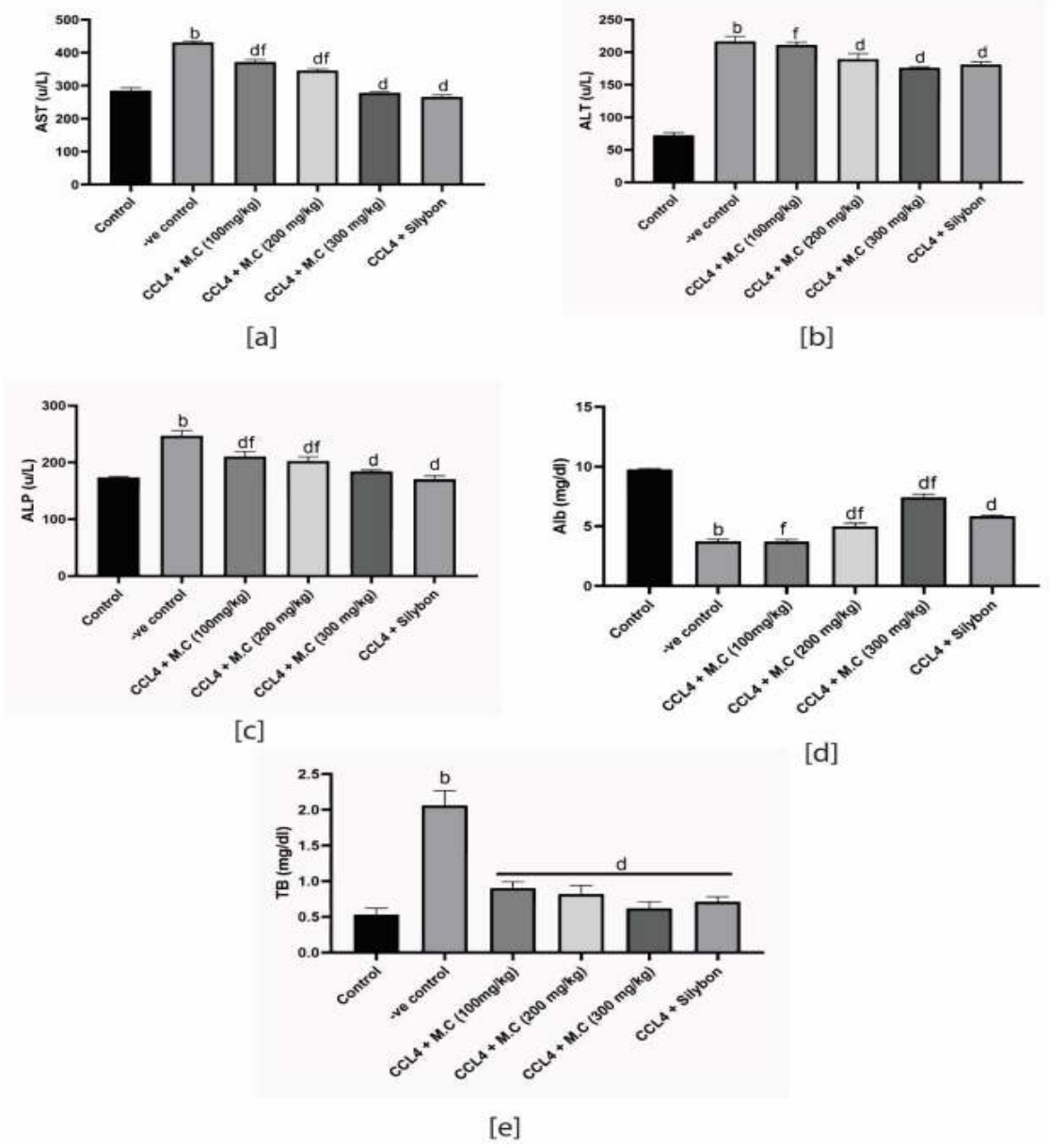
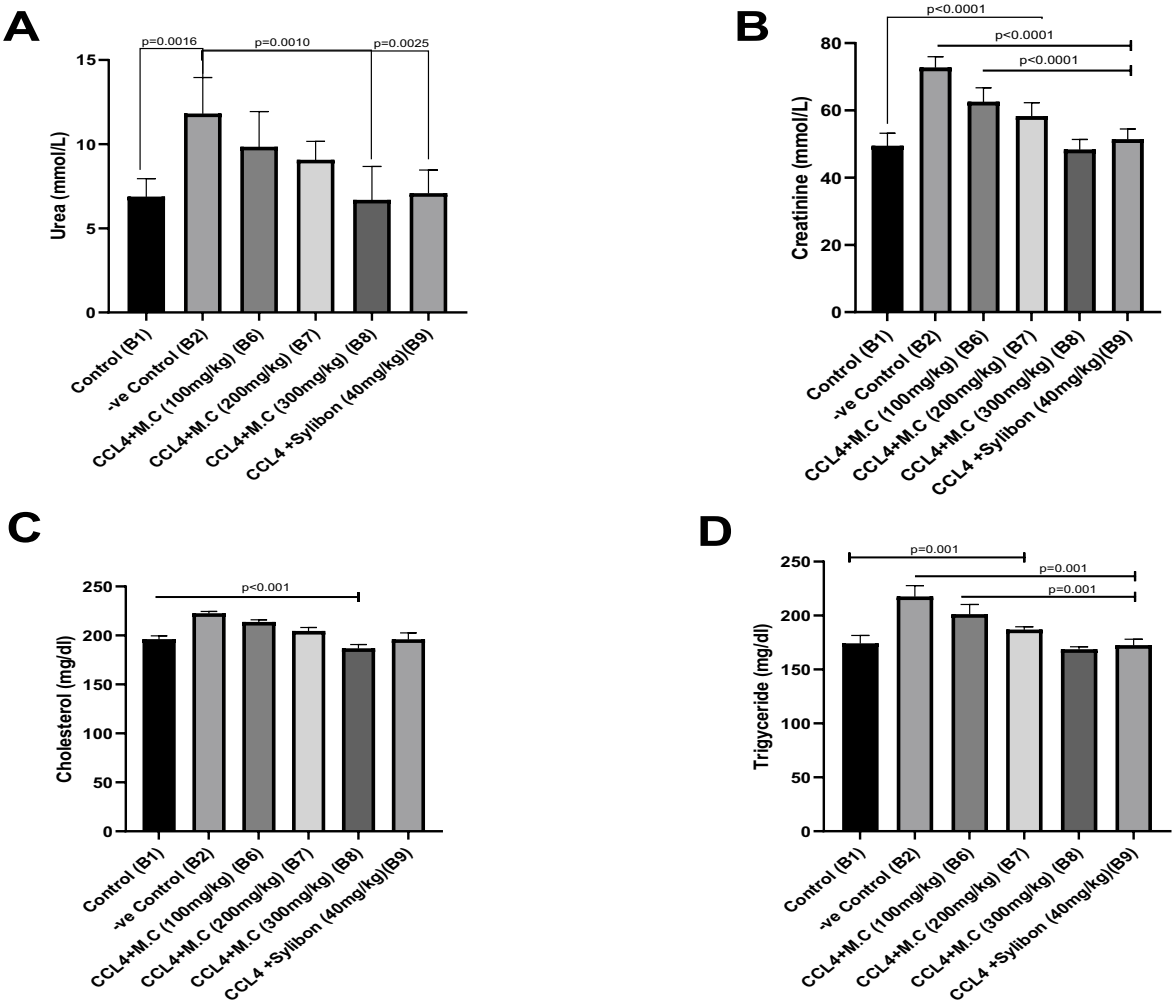


Figure 1: The effect of *M. charantia* extract on the level of liver (a) aspartate aminotransferase (b) alanine aminotransferase (c) alkaline phosphatase (d) albumin and (e) bilirubin in CCl4-induced liver injury in wistar rats. b" represents p < 0.01 to control, "d" represents p < 0.01 to Negative control, and "f" represents p < 0.01 to CCL4 + Silybon (40 mg/kg).

Figure 2: Effect of *M. charantia* on CCl4 induced changes in serum Urea and Creatinine of the Wistar rat. Effects of *Mormodica charantia* on CCL4 induced changes in serum lipid profile in Wistar rats. Figure 2a and 2b showed that there is a significant (<0.05) increase in the level of urea and creatinine in the negative control group compared to the control group. Also, there is a significant (<0.05) decrease in the levels of urea and creatinine in the (100 mg/kg), (200 mg/kg), (300 mg/kg) *M. charantia* and (40 mg/kg) Silybon treatment groups when compared to the negative control group. The result also showed a significant (<0.05) increase in the levels of urea and creatinine in the (100 mg/kg) and (200 mg/kg) *M. charantia* treatment group but a significant (<0.05) decrease in the levels of urea and creatinine in the (300 mg/kg) *M. charantia* treatment group compared to the silybon treatment group.

Figure 2c, 2d, 2e and 2f showed that there is significant (<0.05) increase in the levels of CHOL, TRIG and LDL but a significant (<0.05) decrease in the level of HDL in the negative control group compared to the control group. In *M. charantia* and Silybon there is significant (<0.05) decrease in the levels of CHOL, TRIG and LDL but significantly (<0.05) increase the level of HDL in the treatment groups compared to the negative control group. In comparison with Silybon treatment group, there is significant (<0.05) increase in the levels of TRIG, CHOL and LDL but a significant (<0.05) decrease in the level of HDL in the (100 mg/kg) *M. charantia* treatment group. There is significant (<0.05) increase in the levels of TRIG and LDL in the (200 mg/kg) *M. charantia* treatment group compared to the silybon treatment group. Also, the result showed that there is significant (<0.05) decrease in the levels of CHOL, TRIG and LDL but significant (<0.01) increase in the level of HDL of the (300 mg/kg) *M. charantia* treatment group compared to the silybon treatment group.



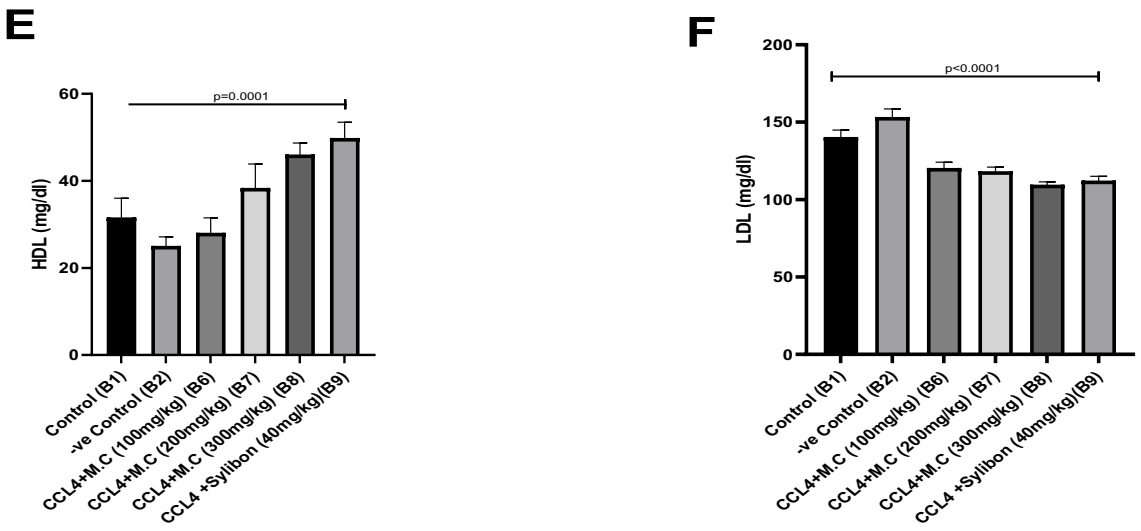


Figure 2: The effect of *M. charantia* extract on the level of liver (a) Urea (b) Creatinine (c) Cholesterol (d) Triglyceride (e) HDL and (f) LDL in CCl4-induced liver injury in wistar rats. b" represents p < 0.01 to control, "d" represents p < 0.01 to Negative control, and "f" represents p < 0.01 to CCL4 + Silybon (40 mg/kg).

Discussion

Liver injury is characterized by the elevated levels of serum hepatic enzymes indicating the cellular leakage and loss of functional integrity of hepatic membrane and structure after CCl4 exposure. High levels of ALT, AST, ALP, total protein, albumin and total bilirubin are sensitive indicators of liver injury and are most helpful in recognizing hepatic diseases because these enzymes are normally localized in the cytoplasm and are released into circulation after cellular damage has occurred (Pradeep *et al.,* 2010; Ku *et al.,* 2013). CCl4 reflects changes in the cell membrane permeability leading to leakage of enzymes from cells to the circulation by showing an increase in the activities of these enzymes (Botsoglou *et al.,* 2008). Increased levels of blood hepatic indicators in a prior study revealed that CCl4 caused a significant liver injury because of an increase in reactive oxygen species, which can harm the liver. This study found that rats exposed to CCl4 had significantly higher levels of serum ALT, AST, ALP and total bilirubin as well as significantly lower levels of total protein and albumin. However, *M. charantia* was able to significantly reverse these effects at doses of 200 mg/kg and 300 mg/kg. In the metabolism of lipids, the liver is essential. Fatty acids are crucial in the etiology of many diseases, including metabolic disorders (Hotamisligil, 2006; Anderson *et al.,* 2009) mainly due to altered level of hepatic lipids, particularly a decrease in polyunsaturated fatty acid (PUFA) and steatosis which is developed in non-alcoholic fatty liver disease. A reduction in fatty acid synthesis, an increase in fatty acid oxidation, and storage of triacylglycerol are all consequences of PUFA's influence on the gene expressions in the liver. According to Arndt *et al.* (2009), the presence of PUFAs may also increase inflammation and steatosis. Free fatty acids are important mediators of lipotoxicity because they operate as potential cellular poisons that might cause an excessive buildup of lipids. According to Unger (2002, Unger and Orci (2002), and Unger (2003), excessive lipid accumulation can enter non-oxidative harmful pathways, causing cell damage and death. Increased levels of free fatty acids have been linked to the severity of nonalcoholic fatty liver disease (Nehra *et al.,* 2001). Lipids are an essential form of energy storage and comprise of fatty acids and their derivatives (Bassendine *et al.,* 2013). The lipoprotein particles comprise of high-density lipoprotein and very low-density lipoproteins which are synthesized and secreted into the circulation by the liver, and the mature particles such as low-density lipoproteins, intermediate-density lipoproteins, chylomicron remnants, and HDL are taken up by the liver in a regulated receptor-dependent manner (Habib *et al.,* 2005). Increase concentration of cholesterol in the liver

is commonly found in chronic liver disease (Ben-Ari *et al*., 2001). A significant decline has been reported in serum cholesterol and triglyceride levels in chronic liver disease (Habib *et al.,* 2005). Exposure to CCl4 caused a significant increase in the triglyceride, total cholesterol, and LDL levels and decrease in HDL level. Increase in the cholesterol levels might be due to the increased esterification of fatty acids, decreased excretion of cellular lipids, and inhibition of fatty acid β-oxidation. CCl4 increases the synthesis of fatty acids and triglyceride from acetate and enhances lipid esterification (Ben *et al.,* 2019; Shaban *et al.,* 2021) and also stimulates the transfer of acetate into liver cells and leads to an increase in cholesterol synthesis. The accumulation of triglyceride in liver might occur due to the inhibition of lysosomal lipase activity and VLDL secretion (Marimuthu *et al.,* 2013). In agreement with these previous studies, this study showed significant increase in the levels of total cholesterol, triglyceride, LDL-cholesterol but significant decrease in HDL-cholesterol after exposure to CCl4. This study also showed a marked decrease in the levels of total cholesterol, triglyceride, LDL-cholesterol and increase in HDL-cholesterol in the *M. charantia* and Silybon treatment groups which is in agreements with previous studies of Hirako *et al*., (2011) and Bougarne *et al*., (2018).

The elevation in the plasma creatinine and urea levels can indicate acute kidney injury (Pourfarjam *et al*., 2017). The exposure to CCL4 also induced renal toxicity evidenced by an elevation of serum creatinine and urea (Abdel *et al.,* 2012; Al-Yahya *et al.,* 2013) and these pathological changes can also be attributed to loss of structural integrity of nephrons (Khan *et al.,* 2010), which is in agreement with reports confirming that the level of serum creatinine increases only if at least half of the kidney nephrons are already damaged (Bellassoued *et al.,* 2018). In addition, decrease in plasma albumin concentration in CCl4-treated rats might have resulted from remarkable leakage due to hyper-cellularity of both glomeruli and tubules. In our study, treatment with *M. charantia* showed a significant decreased in the levels of urea and creatinine. This effect may be related to the antioxidant properties of *M. charantia* since it has been found that ROS may impair glomerular filtration rate (El-mohsen Ali and Abdelaziz, 2014).

In conclusion, the results showed that CCl4 caused liver and kidney damage through hyperlipidemia, inflammation and oxidative stress. However, *administration of M. charantia* demonstrated significant hepatoprotection and renal protection against CCl4 induced liver and kidney injury in Wistar rats. *M. charantia* significantly reduced the inflammatory and oxidative stress markers as well as fatty acid metabolism. These study, thus, suggest that herbal medicine possesses ameliorative and confer protection against liver and kidney toxicity.

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