Protective Effects of *Moringa Oleifera* Extract Against Oxytetracycline-Induced Fatty Liver in Male Rats

Dr. Reham Ragab Abd El Maboud
Home Economics Department, Faculty of Specific Education, Assiut University.

Assistant prof.Mahmoud Ashry Ebrahim
Zoology Department, Faculty of Science, Al-Azhar University, Assiut

Assistant prof.Eman Mahmoud El Serwy
Nutrition and Food Sci. Dept., Faculty of Home Economics, Helwan Univ, Cairo, Egypt

Abstract

Recent years have witnessed a significant increase in the causes of fatty liver disease among Egyptians, including the use of some medications without a doctor's prescription. Currently, the use of medicinal plants has increased as an alternative to chemical compounds due to their low cost and limited side effects. The purpose of this study was to examine the putative potential preventive effects of *Moringa oleifera* Extract (MOE) against Oxytetracycline (OTC) caused fatty liver in an attempt to understand its mechanism of action. 40 of adult male Wistar rats (150-170g) were randomly divided into four groups (10 animals each group) as follows: group (1): negative control group, group (2): fed the basal diet + 300 mg/kg body weight/day) of MOE, group (3): rats infected with OTC (120 mg/kg) for three consecutive days and fed the basal diet, and group (4): rats infected with OTC, fed the basal diet + 300 mg/kg of MOE daily. After consecutive six weeks, the results revealed that the administration of MOE markedly restored the pathophysiological deteriorations resulted from OTC intoxication; This was evidenced by the marked reduction in serum ALT, AST, GGT, ALP, LDH and bilirubin as well as the improvement in the serum level of total protein, albumin, lipid profile and antioxidant assay. Moreover, the microscopic examinations showed marked regeneration of the hepatocytes. In conclusion, MOE could play a beneficial role for prevention of OTC induced pathophysiological distortions. So, it may

be worthy to consider the beneficial use of moringa extract as a supplement with the fatty liver therapy.

Keywords: Moringa oleifera**,** Oxytetracycline, Oxidative stress, Alternative medicine, Liver structure and functions**.**

التأثيرات الوقائية لمستخلص المورينجا أوليفيرا ضد الكبد الدهني الناتج عن الأوكسي تيتراسيكلين في ذكور الفئران

المستخلص:

شهدت السنوات الأخيرة زيادة ملحوظة في أسباب الاصابة بالكبد الدهني بين المصريين, ومنها استخدام بعض الأدوية دون وصف الطبيب. في الوقت الحالي زاد الاتجاه نحو استخدام النباتات الطبية كبديل للمركبات الكيميائية لتكلفتها المنخفضة وآثارها الجانبية المحدودة. لذا كان الهدف من هذه الدراسة هو تقييم التأثيرات الوقائية المحتملة لمستخلص أوراق المورينجا أوليفيرا ضد الكبد الدهني الناجم عن أوكسي تتراسيكلين في محاولة لفهم آلية عملها. تم تقسيم ذكور فئران ويستار البالغة (١٥٠-١٧٠ جم) عشوائيًا إلى أربع مجموعات (١٠ حيوانات في كل مجموعة) على النحو التالي: المجموعة (١): مجموعة ضابطة سالبة، المجموعة (٢): تتغذى على الوجبة الغذائية الأساسية + ٣٠٠مجم / كجم وزن الجسم / يوم) من مستخلص المورينجا، المجموعة (٣): الفئران المصابة بأوكسي تتراسيكلين (١٢٠ مجم / كجم) لمدة ثلاثة أيام متتالية و تتغذى على الوجبة الغذائية الأساسية ، والمجموعة (٤): الفئران المصابة بأوكسي تتراسيكلين, تتغذى على الوجبة الغذائية الأساسية + ٣٠٠مجم / كجم من مستخلص المورينجا يومياً. بعد ستة أسابيع متتالية، كشفت النتائج أن إعطاء مستخلص المورينجا ساهم بشكل ملحوظ في تحسين التدهورات المرضية الفسيولوجية الناتجة عن التسمم بالأوكسيتتراسيكلين؛ حيث لوحظ انخفاض كبير في مستويات انزيمات الكبد (ALT وAST وGGT وALP وLDH والبيليروبين), مع تحسن في مستويات البروتين الكلي والألبومين ودهون الدم واختبارات مضادات الأكسدة. علاوة على ذلك، أظهرت الفحوصات المجهرية تجديدًا ملحوظًا للخلايا الكبدية. تشير هذه النتائج الى أن مستخلص المورينجا يمكن أن يلعب دورًا وقائياً فعالاً ضد التشوهات الفسيولوجية المرضية الناجمة عن الأوكسيتتراسيكلين, مما يعزز من قيمته كمكمل في علاج الكبد الدهني.

الكلمات المفتاحية: المورينجا أوليفيرا، أوكسي تتراسيكلين، الإجهاد التأكسدي، الطب البديل، تركيب الكبد ووظائفه.

INTRODUCTION

The liver is a central organ in the human body, coordinating several key metabolic roles, and plays a major role in the metabolism, detoxification and excretion of xenobiotics ***(Acharya et al., 2021 and Naga, 2023)***. Non-alcoholic fatty liver disease (NAFLD), currently known as metabolic‐associated fatty liver disease (MAFLD), is the most common liver disease worldwide, and it is a leading cause of chronic liver disease globally ***(Cheemerla and Balakrishnan, 2021)***. The clinical condition known as Non-Alcoholic Fatty Liver Disease (NAFLD) is mainly defined by the accumulation of fat in the liver parenchyma (>5% of hepatocytes). It can manifest in a variety of ways, from simple hepatic steatosis to the more aggressive nonalcoholic steatohepatitis (NASH), which culminates in hepatic cirrhosis and hepatic arcinoma through progressive organ fibrosis. Crucially, fibrosis and NASH are essential for the development of cirrhosis and hepatocellular cancer ***(Abenavoli et al., 2016; Chalasani et al., 2018; Powell et al and Loomba et al., 2021).***

The estimated global incidence of NAFLD is 47 cases per 1,000 populations and is higher among males than females (males 40% and females 26%). Over time, the prevalence of NAFLD has grown worldwide, rising from 26% in studies conducted in 2005 or before to 38% in research conducted in 2016 ***(Younossi et al., 2016 and Riazi et al., 2022).*** If current trends continue, it is predicted that the prevalence of NAFLD would rise dramatically in several parts of the world by 2030 ***(Riazi et al., 2022).***

The prevalence of NAFLD varies substantially by world region, contributed by differing obesity rates, and genetic and socioeconomic factors. NAFLD may become more common as a result of rising obesity and type 2 diabetes rates ***(Riazi et al.,2022)***. The main pathological pathway involved in most liver disorders is oxidative stress and its associated lipid peroxidation ***(Naga, 2023).*** A major contributing factor to the development of NAFLD is obesity. Notably, metabolically unhealthy lean (MUL) people can also be diagnosed with NAFLD. Despite their lean physique, the role of adipose tissue dysfunction in contributing to NAFLD in individuals with MUL should not be overlooked, as it stems from a limited capacity for adipose tissue expansion ***(Lee et al.,2023).***

Over the past few decades, Egypt's chronic liver disease landscape has seen a significant shift, with the incidence of NAFLD rising to alarming proportions and the prevalence of viral hepatitis declining, resulting in a significant burden on both individuals and healthcare systems ***(Fouad et al., 2022)***. Energy intake has increased generally in the Egyptian population's nutritional pattern during the last 50 years. Nutrition shifted to a diet that included less consumption of fresh fruits

and vegetables and more consumption of processed foods, fast food, red meat, vegetable oils, and soft beverages ***(Golzarand et al., 2012).*** Up to 40% of the fat that Egyptian women consume are believed to be saturated fat, and 80% of them are thought to consume less than five servings of fresh fruit and vegetables each day ***(Mahmood et al., 2010 and WHO, 2014).***

The excessive and unregulated use of Oxytetracycline (OTC) without medical supervision has been shown to have harmful effects on the liver and kidneys ***(Oda et al., 2018).*** OTC, a tetracycline antibiotic, is highly effective against a broad range of microorganisms, including Gram-positive and Gram-negative bacteria, as well as *Chlamydia*, *Rickettsia*, *Mycoplasmas*, and protozoan parasites ***(Nelson and Levy, 2011).*** It is widely prescribed for treating respiratory and skin diseases in humans and livestock, particularly in developing countries, due to its antimicrobial effectiveness and affordability. Previous studies have reported that toxic doses of OTC can lead to severe microvesicular steatosis and even hepatic damage ***(Saraswat et al., 1997).*** Additionally, OTC has been found to induce hepato-renal toxicity by triggering membrane lipid peroxidation and depleting antioxidant biomarkers in tissues ***(Gnanasoundari and Pari, 2006)***.

Inspite of tremendous advancements in modern medicine, natural antioxidants and hepatoprotective agents are widely considered to be superior over conventional drugs due to the adverse effects associated with the latter ***(Naga, 2023).*** In recent years, there has been growing interest in using herbal remedies to treat various diseases ***(Hussain et al., 2019).*** Natural medicinal plants are widely used globally and played a vital role in healthcare. This is because they are cheap, easily accessible, simple to consume, generally safe, and highly effective ***(Patil and Gaikwad, 2010 and Madi et al., 2016).***

Moringa oleifera (MO) belongs to the family *Moringaceae* which can be cultivated in any tropical or subtropical areas ***(Thurber and Fahey, 2010)***. It is a rapidly growing tree that reaches a height of approximately 10 meters. Native to India, this plant is commonly found in tropical regions and grows in all soils ***(Kesharwani et al., 2014)***. MO is one of the most important natural plants which used as food and medicine because they are good sources of bioactive compounds including nutrient and anti-nutrient substances ***(Gopalakrishnan et al., 2016)***.

These nutrients are used to combat malnutrition ***(Mahmood et al., 2010 and Sahay et al., 2017)***. Leaves of MO have a high nutritive value as they are rich in minerals such as potassium, calcium, magnesium, iron, zinc and copper ***(Kasolo et al., 2010)***. They are also rich in vitamins like

vitamin A, folic acid, vitamin B6 and vitamin B3, ascorbic acid, vitamins D and E ***(Mbikay, 2012)***. In addition, MO leaves also contain proteins, fibers, phytochemicals like sterols, tannins, flavonoids, terpenoids, anthraquinones, saponins, alkaloids and glucosinolates, isothiocyanates, glycoside compounds ***(Berkovich et al., 2013 and Dhakad et al., 2019).*** This renders MO leaves to have anticancer, anti-inflammatory, anti-ulcer, antihypertensive, antibacterial and immunostimulatory properties ***(Ijarotimi et al., 2013and Jung, 2014).*** The present study was conducted to evaluate the beneficial effect of *moringa* aqueous extract (MOE) on fatty liver status induced by oxytetracycline (OTC) in albino rats.

MATERIALS AND METHODS

Materials

Source of plant

Dried *Moringa oleifera* leaves were obtained from a local supplier, Abd El-Rahman Harraz, located in the Bab El-Khalk area, Cairo, Egypt, in 2024**.**

Chemicals and kits

Oxytetracycline was acquired from the Medical Union Pharmaceutical Company (MUP) in Egypt, while all other chemicals, which were of analytical grade, were sourced from Sigma (St. Louis, USA) and Fluka (Buchs, Switzerland), Human Gesell Schaft fur Biochemical und Diagnostic mbH; DiaSys Diagnostic systems GmbH; Diamond Diagnostics, MDSS GmbH Schiffgraben 4130175 Hannover, Germany and Biodiagnostic, Dokki, Giza, Egypt

Experimental rats

Forty adult male albino rats, weighing (150-170g), were obtained from the Animal Colony, National Research Centre, Egypt.

Ethical approval

The researchers got the approval of the Ethical Committee, Faculty of Science (NO. AZHAR 9/2024) , Al-Azhar University, Assiut, Egypt, and it complies with the International Guidelines for Research Ethics.

Methods

Moringa oleifera extraction

This study focused on the aqueous extract of the herb instead of those extracted with organic solvents, as organic solvents might alter the structure and configuration of the extract components. A specialist botanist from Cairo University's Faculty of Pharmacy recognized the herb scientifically and determined that it had a taxonomy serial number (TSN 503874). ***Berkovich et al. (2013)*** procedure was followed while

performing the aqueous extract. After soaking 50 g of dry herb leaf powder in 500 ml of boiling distilled water for three hours, the mixture was lyophilized using a freeze dryer from Snijders- Scientific in Tilburg, Holland, and filtered using sterile Whatman filter paper number 42 (Whatman International Ltd., Maidstone, England).

Chemical composition of *Moringa* leaves powder.

Moisture, protein, ash, fat, crud fiber and minerals were determined according to the methods outlined in the ***AOAC (2016)*** official methods. Total carbohydrates were calculated by difference as mentioned by ***Abd El-Latif, (1990)*** according to the following equation: Total carbohydrates =100 – [moisture (%) + crude protein (%) + crude fat (%) + ash (%)]. While total energy was calculated as mentioned by ***Merrill & Watt, (1955)*** according to the following equation: Energy (kcal) = [4 × (g protein + g carbohydrates)+ 9 × g fat].

Determination of total extract yield

The filtrate was moved into a quick-fit round-bottom flask with a known weight (W1), freeze-dried, and then reweighed (W2). The yield was calculated using the formula:

$$\text{Yield (g/ g crude herb)} = \frac{W2-W1}{W3}$$

W1, W2, and W3 are the weights of the clear and dry quick-fit flask, the flask containing the extract after lyophilization, and the crudely powdered herb, respectively, in grams ***(Muhamman et al., 2013).***

Determination of total phenolics

The total phenolic content in the aqueous extract was analyzed spectrophotometrically using the modified Folin-Ciocalteu colorimetric method of ***Jayaprakasha and Rao, (2000).***

Determination of radical scavenging activity (RSA)

Radical scavenging activity (RSA) was determined by assessing the ability of antioxidants in the extract to neutralize the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical using the method of ***Nogala-Kalucka et al., (2005)***.

Experimental design

Animals were maintained on free access to food and water for a week before starting the experiment for acclimatization. After being acclimatized, the animals were divided randomly into four groups (10 animals each); group (1): negative control group, group (2): fed on basal

diet + 300 mg/kg body weight/day) of MOE, group (3): rats infected with OTC (120 mg/kg) for three consecutive days and fed on a basal diet as a positive control group, and group (4): rats infected with OTC and fed on a basal diet + 300 mg/kg of MOE daily. MOE dose was determined based on ***Jaiswal et al. (2009),*** while TOC dose was according to ***El-Deab & Alamer (2021).***

Blood and tissue sampling

Blood and tissue samples were collected after six weeks, marking the end of the treatment period. Rats were weighed and fasted overnight, and blood samples were drawn from the retro-orbital plexus using sterile, heparinized glass capillaries after the rats were anesthetized, allowed to clot for 20 minutes, and then cool-centrifuged for 10 minutes at 3000 rpm; the sera were separated, divided into aliquots, and stored at -80°C till biochemical measurements were carried out as soon as possible. Immediately after blood collection, the animals were sacrificed; then the liver of each animal was dissected; one part of the liver was washed in saline, dried, rolled in a piece of aluminum foil, and stored at -80 °C for determination of biochemical measurements; the second part was soaked in formaldehyde-saline (10%) mixture buffer for histopathological processing and microscopic examination.

Tissue homogenization

10% homogenate (w/v) was obtained by homogenizing a liver specimen in ice-cold phosphate buffer (50 mM, pH 7.4). To eliminate the cell/ular ghosts and nuclear and mitochondrial fractions, the homogenate was centrifuged for 20 minutes at 10,000 rpm. The supernatant was then divided into aliquots and kept at -80°C until the biochemical analyses could be completed.

Biochemical determinations

The activities serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of ***Reitman and Frankel, (1957).*** Alkaline phosphate (ALP), GGT and lactate dehydrogenase (LDH) were determined according to ***Dumas et al., (1971), William (1980) and Szasz et al., (1974).*** Total proteins, albumin and bilirubin level were estimated according to ***Weichselbaum (1946), Eastham (1976)*** **and** ***Walter and Gerade, (1970),*** respectively. Total cholesterol, triglycerides, LDL, HDL and VLDL (very low density lipoprotein) concentrations were measured according to ***Richmond (1973), Lopes-Virella et al. (1977), Fossati & Prencipe (1982), Assmann***

(1979), and Warnick & Albers (1978). respectively. Hepatic MDA as well as CAT activities were estimated according to ***Ohkawa et al. (1979).***

Hepatic histopathology investigation

Paraffin sections, each 5µm thick, were stained with hematoxylin and eosin ***(Drury and Wallington, 1980)*** and examined under a light microscope.

Statistical analysis

Analysis of variance (one way ANOVA), and the significance of differences among means were assessed using the Waller-Duncan k-ratio test ***(Waller and Duncan, 1969)***. Significance was determined at a p-value of ≤ 0.05. The test was carried out using statistical analysis system (SAS) program software.

RESULTS AND DISCUSSION

1- Chemical composition of *Moringa oleifera* leaves (MOL)

Table (1) demonstrates the contents of MO leaves in terms of moisture, ash, crude fat, protein, crude fiber, and total carbohydrates. All results were calculated as (g/100g on dry weight), carbohydrates were calculated by difference. The results showed that crude protein (about 23.09%), crude fiber (6.25%), ash (12.6%), carbohydrates (about 41.03%), moisture (10.53%), and crude fat (12.75%).

Table (1): Chemical composition of MOL on a dry weight basis (mean ± SD) (g/100 g)

Sample	Moisture %	Ash %	Crude fat %	Protein %	Crude fiber %	Carbohydrate %	Energy kcal
MOL	10.53±0.11	12.60±0.17	12.75±0.08	23.09±0.29	6.25±0.19	41.03±0.08	371.23±0.19

- Mean three replicates
- MOL: *Moringa oleifera* leaves
- % Protein = % Nitrogen × 6.25
- Total carbohydrate = 100 – (Moisture +Ash+ Crude fat + Protein)
- Energy = (4x protein) + (9 x fat) + (4 x carbohydrates) in grams

The results of this study showed that MO is an important plant with leaves that have high concentrations of energy and nutrients. MO is reported as a good source of six major nutrients; carbohydrates, especially dietary fibers, proteins, vitamins, minerals, lipids, and water. The unique features of *Moringa oleifera* are its richness in proteins, carbohydrates, and fibers with low fat ***(Moyo et al., 2011).*** Carbohydrates serve as the primary source of energy. The ash content of approximately 12.6% shows that the leaves are high in mineral elements. MO leaf powders are a great food source, as demonstrated by the chemical

composition values, which support their direct use in human nutrition or the creation of balanced diets for animal nutrition ***(Valdez-Solana et al., 2015).***

The obtained results are closely pertinent to observed results by ***Amaby and Gebrehiwo (2015); Barakat and Ghazal (2016), and Ntshambiwa et al., (2023).*** But there are variations in obtained results with ***Sengev et al., (2013); Abo-Rhyem (2018) and Peñalver et al., (2022),*** the variability of different contents in macronutrients and micronutrients among the studies observed can be explained by a difference in the soil composition that can influence the soil nutrient absorption by the plants. Indeed, the soil factors act on the mineral composition and can modify the soil composition and the nutritional properties, or they act on the plants' absorption ***(Heller et al., 1998).***

2- The yield, TPC, and RSA of MOE

Figure (1) shows the yield, total phenolic content (TPC), and radical scavenging activity (RSA) of the *Moringa* aqueous extract. According to the results gathered, the aqueous extract of MO has a high phenolic component concentration and a high RSA, which is one of the antioxidant mechanisms.

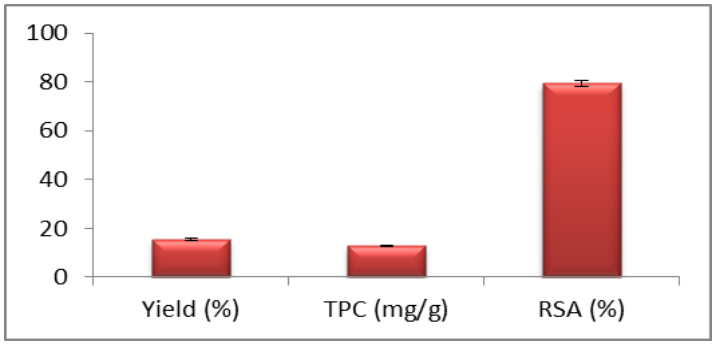

Figure (1): Yield, TPC, and RSA of MOE

3- Body weight gain of groups

The OTC-intoxicated group's body weight gain was significantly lower than the control group, suggesting severe disruptions in the body's physiology, assimilation, and metabolism, while the MOE-treated group's body weight gain remained constant from the control group, confirming that MOE has no adverse effects on the animals' metabolic rates. Positively, MOE administration in conjunction with OTC considerably reduced the decline in body weight progression, demonstrating MOE's high effectiveness and ability to bring body weight back to a level that is somewhat within control. **(Figure 2).**

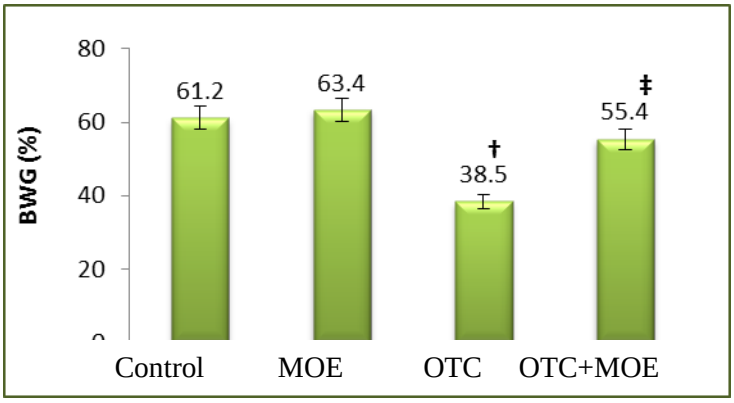

Figure (2)): Body weight gain (%) of experimental groups

- (†) significant from the control group, (‡) is significant from the OTC group.

4- Effects of *MOE* against OTC induced fatty liver in male rats on ALT, AST, ALP, GGT, and LDH activity

Table (2) indicated that OTC administration significantly increased bilirubin, AST, ALT, ALP, GGT, and LDH activity, but MOE alone had no detrimental effects on these parameters as compared with those of normal rats. The OTC-induced alterations in the measures were considerably lessened when OTC was administered with MOE.

Table (2): ALT, AST, ALP, GGT, and LDH activity (mean ±SE)

Groups / Parameters	Control (-)	MOE	OTC	OTC + MOE
ALT (U/L)	53.7 ± 4.1^{B}	49.3 ± 2.4^{B}	115 ± 4.6^{A}	59.5 ± 3.8^{B}
AST (U/L)	116 ± 1.5^{C}	104.5 ± 4.4^{C}	219 ± 13.8^{A}	127.4 ± 5.5^{BC}
GGT (U/L)	5.13 ± 0.5^{C}	5.1 ± 0.2^{C}	14.2 ± 0.2^{A}	6.8 ± 0.5^{BC}
ALP (U/L)	220.3 ± 22.5^{C}	212.3 ± 10.1^{C}	367 ± 19.3^{A}	233.6 ± 18.4^{BC}
LDH (U/L)	1390 ± 58^{C}	1228 ± 75^{C}	2195 ± 13.8^{A}	1639 ± 26.5^{BC}
T. bilirubin(mg/dl)	0.136 ± 0.008^{B}	0.125 ± 0.002^{B}	0.88 ± 0.077^{A}	0.25 ± 0.028^{B}
D. bilirubin(mg/dl)	0.029 ± 0.0005^{C}	0.028 ± 0.002^{C}	0.20 ± 0.010^{A}	0.038 ± 0.003^{B}

- SE: standard error.
- The means in each row that have different letters in superscript are substantially different at $p\leqslant0.05$.
- MOE (*Moringa oleifera* extract) - OTC (Oxytetracycline).

This study showed that OTC-induced fatty liver treatment caused a significant increase in serum levels of ALT, AST, GGT, ALP, and LDH activity; these findings agree with previous reports of ***Iida et al. (2022) and Zhou et al., (2023).*** As these enzymes are normally present in the cytoplasm of the hepatocyte and are released into the circulation following disruption of cell-membrane permeability due to oxidative stress and lipid peroxidation caused by the cytotoxic effects of OTC, this

elevation may be the result of structural damage to the hepatocyte. Additionally, there was an elevated serum concentration of alkaline phosphatase, a specific indicator of biliary epithelium affection, which may reflect compression of intrahepatic biliary canaliculi by inflammatory cells in portal tracts ***(Zhou et al., 2023).***

The observed leakage of cellular ALT, AST, GGT, ALP, and LDH activity into the circulation that suggests hepatocellular damage can be explained by OTC doses increasing the oxidative stress markers in the liver as indicated later by the high level of MDA and the significant depletion in CAT level when compared to the normal control group ***(Helal et al., 2011).***

There was a significant decrease in ALT, AST, ALP, GGT, and LDH activity as well as improvement of antioxidant and oxidative stress parameters when treated with OTC drug combined with MOE. It could be because of hepatoprotective activity. The stabilizing effect of the cell membrane to stop enzyme leaks, as previously hypothesized ***(Pari and Karthikesan, 2007)***, maybe the reason for the reversal of elevated serum intracellular enzyme levels by MOE extract. An earlier study found that the presence of quercetin and kaempferol was responsible for the hepatoprotective effect ***(Selvakumar and Natarajan, 2008).*** MOE prevents the permeation of toxins by contending for the same receptor sites on cell membranes. This could be caused by a combination of two main mechanisms: 1) a change in cell membranes that makes it possible for very small amounts of toxins to enter the cell; 2) an acceleration of protein synthesis that stimulates cellular regeneration ***(Buraimoh et al., 2011).***

5- Effects of *MOE* against OTC induced fatty liver in male rats on total proteins, albumin, globulin, and A/G ratio rats

Compared with the control group, the current study demonstrated that rats given MOE orally daily experienced non-significant changes in serum total proteins, albumin and globulin levels, and the A/G ratio, whereas intoxication with OTC resulted in a significant decrease in these protein profiles. However, rats given MOE in addition to OTC orally for six weeks showed a significant improvement in that protein profile (Table 3).

Table (3): Total proteins, albumin, globulin, and A/G (mean ±SE)

Groups / Parameters	Control (-)	MOE	OTC	OTC + MOE
T.Protein (g/dl)	8.5 ± 0.02^{A}	8.56 ± 0.08^{A}	5.9 ± 0.02^{C}	7.9 ± 0.03^{AB}
Albumin (g/dl)	4.3 ± 0.02^{A}	4.46 ± 0.05^{A}	2.8 ± 0.028^{B}	4.2 ± 0.054^{A}

Globulin (g/dl)	4.1 ± 0.008^{A}	4.2 ± 0.051^{A}	3.0 ± 0.011^{C}	3.7 ± 0.02^{B}
A/G Ratio	1.0 ± 0.011^{A}	1.0 ± 0.017^{A}	0.9 ± 0.008^{B}	1.1 ± 0.02^{A}

- SE: standard error.
- The means in each row that have different letters in superscript are substantially different at $p \leqslant 0.05$.
- MOE (*Moringa oleifera* extract) - OTC (Oxytetracycline).

Treatment with MOE significantly increased serum total protein concentrations, which may be associated with a reduced affinity for albumin. In rats treated with MOE and its active compounds (moringinine, quercetin, and chlorogenic acid), the two parameters normalized, showing inhibition of liver cell damage and a reduction in the leakage of enzymes into the blood, which is consistent with a recent study that found that MOE reduced toxicity due to the elimination of the toxic products of OTC in rats ***(Okumu et al., 2016)***. The liver-protective effects of herbal plants are primarily due to their chemical constituents, which include phenols, coumarins, lignans, essential oils, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids, and xanthene. These compounds, found in *M. oleifera*, are key to its liver-protective action ***(Buraimoh et al., 2011).***

6- Effects of *MOE* against OTC induced fatty liver in male rats on cholesterol, triglyceride, HDL, LDL and VLDL levels.

The results of the lipid profile in Table (4) illustrated that rats given MOE also showed no adverse effects on serum total cholesterol, triglycerides, LDL, HDL, and VLDL. However, when comparing the two groups to the corresponding values of the control group, OTC intoxication significantly raised total cholesterol, triglycerides, LDL, and VLDL while significantly lowering HDL levels. Thankfully, MOE and OTC-fed mice demonstrated a considerable decrease in cholesterol, triglycerides, LDL, and VLDL as well as a clear increase in HDL.

Table (4): Serum cholesterol, triglyceride, HDL, LDL and VLDL levels of experimental groups (M±SE)

Groups / Parameters	Control (-)	MOE	OTC	OTC + MOE
Cholesterol (mg/dl)	157 ± 7.5^{C}	153 ± 1.15^{C}	221.6 ± 2.5^{A}	155.7 ± 1.7^{B}
Triglyceride (mg/dl)	116 ± 5.7^{C}	115.6 ± 0.8^{C}	265 ± 13.1^{A}	136.5 ± 5.4^{B}

LDL-c (mg/dl)	43 ± 1.4^{C}	43.5 ± 0.28^{C}	97.5 ± 0.28^{A}	42.5 ± 0.29^{B}
HDL-c (mg/dl)	90.8 ± 5.7^{A}	86.2 ± 0.69^{AB}	71.1 ± 10.1^{C}	85.9 ± 6.2^{AB}
VHDL-c (mg/dl)	23.2 ± 1.1^{C}	23.1 ± 1.02^{C}	53.0 ± 1.3^{A}	27.3 ± 1.09^{B}

- M±SE: mean ±standard error;
- Within each raw, means with superscript different letters are significantly different at $p\leq0.05$
- MOE (*Moringa oleifera* extract) - OTC (Oxytetracycline).

OTC-intoxicated rats showed a substantial increase in blood TG, cholesterol, bilirubin, and lipid peroxidase levels, while the OTC-induced fatty liver caused a decrease in total protein levels. The reduction is ascribed to the initial damage that is produced and located in the endoplasmic reticulum. This damage causes P450 to be lost, which prevents it from functioning properly and resulting in a drop in protein synthesis. Additionally, the accumulation of TG causes fatty liver. Another effect of intoxication was the inhibition of bile acid production from cholesterol, which raised cholesterol levels. The MOE-induced suppression of cholesterol levels indicates that the inhibition of bile acid production was reversed. OTC-intoxicated rats are more vulnerable to hepatotoxicity because of a reduction in their antioxidant defense systems ***(Rahate and Rajasekaran, 2015)***

7- Effects of *MOE* against OTC induced fatty liver in male rats on hepatic oxidative markers MDA and CAT activity

The results obtained in Figure (3) regarding the control group showed no adverse changes in the hepatic oxidative markers (MDA and CAT). Although the intoxication with OTC resulted in marked elevations in MDA and a significant depletion of CAT activity. Supplementing the animals with MOE in addition to OTC caused a marked decrease in the oxidative stress (MDA) concurrent with a significant improvement in the anti-oxidative battery CAT activity compared to the OTC group.

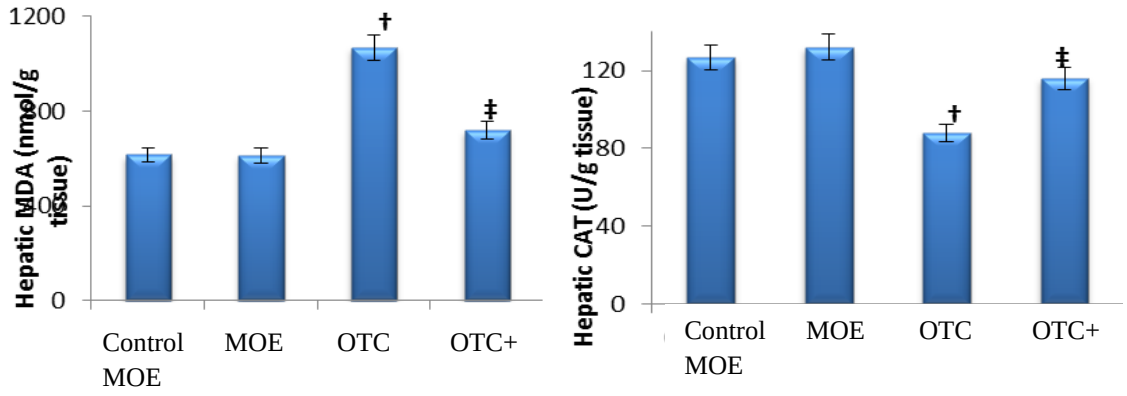

Figure (3): Hepatic MDA and CAT of experimental groups

- (†) significant from the control group, (‡) significant from the OTC group

Additionally, lipid peroxidation was significantly reduced in MOE-treated groups compared to OTC groups, as indicated by a decrease in MDA levels and an increase in antioxidant enzyme activity as CAT. These findings support the antioxidant effect of MOE and are consistent with research conducted by ***Essawy et al., (2017); Qasim & Baraj, (2017)*** *and* ***Ashry et al. (2019).***

OTC with MOE reduced lipid peroxidation and increase CAT activity, which may be due to the presence of flavonoids like kaempferol and quercetin, vitamin A, and ascorbic acid, which is a powerful antioxidant. ***Islam and Alam,(2019)*** found similar results.

Hepatic histopathology investigation

Examination of H and E-stained sections of control rats revealed that the liver appeared to be composed of hexagonal classic lobules. Each lobule was traversed centrally by a central vein. The parenchyma of these lobules was composed of liver cells (hepatocytes). The hepatocytes appeared to be arranged in the form of branching cords that radiated from the central veins to the periphery of the classic hepatic lobules. These cords were separated by blood sinusoids, which were lined by flat endothelial cells (Figure 4A). The treatment of rats with MOE at dose level 300 mg/kg/day showed normal architecture (Figure 4B). Histological changes in the liver of rats treated with OTC at a dose level of 120 mg/kg demonstrated that loss of hepatocellular architecture with vacuolar and fatty changes (steatosis) and dilated blood sinusoid. Fibrosis, thick fibrous bands formed of many fibroblast and collagen fibers. The bands run in septa between the hepatocyte nodules, form pseudo lobules, and extend around the blood vessels (Figure 4C). The group of rats treated with MOE companied OTC showed more improvement in deleterious effects induced by OTC in the form of no

vacuolar and fatty changes and no hepatocyte degeneration although moderate fibrous tissue, especially around blood vessels still present (Figure 4D).

In this study, the OTC group experienced immediate pathological alterations in their livers, including necrosis and restricted blood sinusoidal lumina as a result of the enlarged, fat-filled hepatocytes. The significant rise in serum cholesterol, triglycerides, and LDL cholesterol is consistent with this conclusion. The fatty degeneration of the liver is associated with an increase in these blood parameters ***(Helal et al., 2011).*** According to ***Asha et al. (2007),*** the biochemical mechanism underlying OTC toxicity is based on mitochondrial damage.

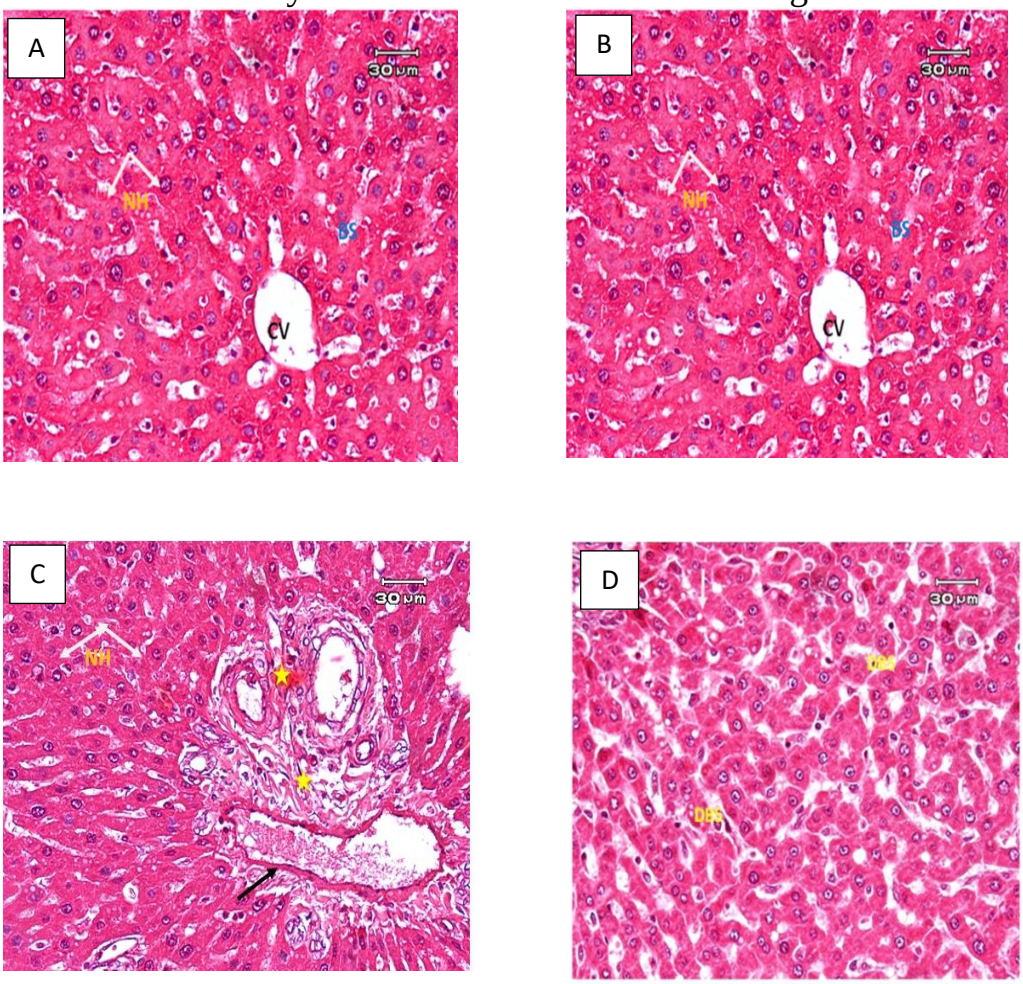

Figure 4(A-D). Photomicrograph of liver section of adult male rats: (A) The control group showing normal hepatic lobule structure with the central vein (CV), hepatic cord, binucleated cell, and hepatic sinusoids (arrowhead) with Kupffer cells. (B) MOE-treated group showing normal hepatic lobule structure with a central vein. (C) OTC-treated group showing hepatocellular architecture with vacuolar and fatty changes (steatosis) and dilated blood sinusoid. (D) OTC+MOE treated group showed reduced amyloid cytoplasmic deposits and improved nuclear appearance.

Conclusion

It is possible to draw the conclusion that MOE protects rats from OTC-induced fatty liver. MOE may mitigate OTC-induced tissue damage by its antioxidant activity, scavenging of free radicals, heightened antioxidant defense system activity, and membrane-stabilizing qualities. Given its extensive use in traditional medicine and additive therapy for a variety of ailments, MOE may be a viable option for a safe supplemental agent for fatty liver disorder.

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