Egyptian Journal of Nutrition
Official journal of the Egyptian Nutrition Society
Print ISSN: 1687-1235
Online ISSN: 2090-2514
Vol. 39 (4): 88 – 105 (2024)
https://ejn.journals.ekb.eg/

The therapeutic and preventive effect of Milk Thistle and Moringa leaves in CCl_4 induced hepatotoxicity in rats

Asmaa M.I.El Gamel[1] and Esraa A. Awaad[2]

[1] Assistant Professor of Nutrition and Food Science, Ahmed Maher Teaching Hospital, Egypt
[2] Assistant Professor of Nutrition and Food Science, Department of home economics, Faculty of Specific Education, Zagazig University, Egypt
[*]Corresponding Author: hala.awaad@yahoo.com

ABSTRACT

The current study was conducted to study the preventive and therapeutic effect of milk thistle and moringa leaves powder on rats suffering from carbon tetrachloride- induced hepatotoxicity. Sixty rats were used for experiments, and the rats were divided into two groups, preventive and therapeutic. The rats were fed different proportions of milk thistle and moringa leaves powder (7.5 and 10%). The following analysis was performed: chemical composition, active ingredients, liver function, kidney function, lipid profile, oxidative stress enzymes for liver tissue and histopathological analysis of the liver. The results of the study were as follows: moringa contains a high percentage of total phenols and flavonoids compared with milk thistle contain, while milk thistle contains a higher percentage of antioxidants compared with moringa. Groups 6 and 12 recorded the best results in all tests under study, and a noticeable improvement was noted in liver tissue in the histopathological study. Therefore, the study recommends consuming moringa and including it in the daily regimen to improve liver function

Keywords: Milk Thistle, Moringa, liver injury**,** Biochemical analysis, Histopathology

Received: 31-12-2024 ***Accepted:14-1-2025*** ***Published: 12-2024***

INTRODUCTION

Liver disease is any injury that affects the liver's capacity to operate. This general term includes all potential problems that lead to the liver's incapacity to perform its assigned duties **Guan and He, (2013).** *Silybum marianum (L.)*, a member of the *Asteraceae*/Compositaceae family, is perhaps the oldest and most researched plant for liver disease treatment **Milić *et al.*, (2013).** Milk thistle seeds, primarily due to their active compound silymarin, are considered to have therapeutic effects mainly focused on protecting the liver from damage caused by toxins, acting as a potent antioxidant, and potentially aiding in liver regeneration by stimulating new cell growth; this makes

it a commonly used herbal remedy for various liver conditions like cirrhosis and hepatitis, while also showing potential benefits for managing blood sugar levels and alleviating symptoms of indigestion. The most important of these components are phenolic chemicals, flavonoids, tannins, and alkaloids. *Silybum marianum (L.)*, often known as milk thistle (MT), is plant of the *Asteraceae* family that originated in the Mediterranean region **Bijak., (2017).** Milk thistle is a medicinal plant with anti-oxidant, anti-inflammatory, anti-fibrogenic, and hypolipidemic properties **Mohammadi *et al*., (2020).**

Silymarin (SIL) is used extensively in European nations to prevent cirrhosis, jaundice, gallstones, and hepatitis, among other hepatobiliary issues. The flavanolignan *silymarin* (SIL) is isolated from the seeds and fruits of the *Silybum marianum*. plant **Choksi *et al*., (2000).** Antioxidant, lipid-lowering, hepatoprotective, antihypertensive, anti-diabetic, anti-atherosclerotic, and anti-obesity properties have been demonstrated for milk thistle **Taj mohammadi *et al*., (2018).**

Okwari *et al*. (2013) showed that *Moringa oleifera* (MO) is one of the most widely cultivated species in the genus Moringa and family *Moringaceae*. Almost every component of *Moringa oleifera* can be eaten as food, and extracts from all parts of the plant exhibit pharmacological qualities that are acknowledged by both the scientific community and common usage, making it one of the most beneficial trees in the world **Oliveira *et al*., (1999).** Almost all of the parts of MO leaves, such as the root, bark, gum, leaf, fruit, flowers, seed, and seed oil, have been used for a variety of biological activities, such as liver disease, hepatic and renal function, thyroid hormone status regulation, antitumor and oxidative stress protection, inflammation treatment, liver protection against various hepatotoxic drugs and carbon tetrachloride, liver and kidney cholesterol reduction and anti-diabetic and hepatoprotective properties **Luka *et al*., (2013) and Ezejindu *et al.*, (2013).**

Moringa leaf powder has numerous pharmacological qualities, including hepatoprotective, anti-inflammatory, and anticancer effects **Lina *et al*., (2023).** Additionally, MO leaf extracts in methanol and chloroform demonstrated a strong defense against albino rats' liver damage caused by CCl_4. In addition to MO leaves, the plant's roots and flowers also have potent hepatoprotective properties. Quercetin, a well-known flavonoid found in moringa flowers, may be the cause of the plant's strong hepatoprotective effects **Selvakumar and Natarajan, (2008).**

Moringa has cardiovascular, hepatoprotective, anti-inflammatory, antibacterial, antioxidant, anticancer, diuretic, antiurolithiatic, and antihelmintic properties **Fozia Farooq *et al*., (2012).**

Numerous research projects are still underway to identify novel, side-effect-free medications for the treatment of liver ailments. Hepatotoxicity can be effectively and safely treated using natural treatments, primarily derived from traditional herbs. Plant-based extracts have also been studied for their antioxidant and hepato-protective properties against liver injury. One of the herbal plants with a broad range of therapeutic uses is *Moringa oleifera* **Yousef *et al*., (2010).** Extracts from several sections of *Moringa oleifera* have been shown to have hepato-protective properties against liver injury in male rats in previous research **Sreelatha and Padma, (2010).** Antipyretic, antioxidant, anti-inflammatory, anti-aging, antidiabetic, antihypertensive,

immunomodulatory, hepatoprotective, and diuretic As a result of active compounds in the bioactive components of *Moringa oleifera* **Elghandour *et al*., (2023).**

This study was conducted to evaluate the potential advantage that can be obtained from using both milk thistle powder and moringa leaves as a therapeutic and preventive effect on rats suffering from hepatotoxicity

MATERIALS AND METHODS

Materials:

- Dried Milk Thistle powder and Moringa leaves powder were bought from National Research Center in Dokki, (Cairo, Egypt).
- Carbon tetrachloride, Paraffin oil, choline, vitamins, minerals, cellulose and casein were bought from El-Gomhoreya Company, Cairo, Egypt.
- Corn starch and oil were bought from a local market, Cairo, Egypt.
- Sixty albino male rats (*Sprague Dawley Strain*) weighing between 150-170g were obtained from Food Technology Research Institute, Giza.

Methods:

Chemical analysis

Chemical analysis of the Milk Thistle powder and Moringa leaves powder including moisture; fat, protein; ash and crude fiber were conducted in Food Technology Research Institute according to the method described by the **A.O.A.C., (2005)**. Carbohydrate value was calculated according to **FAO, (1982)** by as follows:

Soluble carbohydrates (%) = 100 – (moisture % + fat % + protein % + ash % + crude fiber %).

Total flavonoid content in Milk Thistle powder and Moringa leaves powder according to **John *et al*., (2014),** Total phenolic and Antioxidant activity content in Milk Thistle powder and Moringa leaves powder were determined according to **Su & Chien (2007).**

Biological Experiment

To reduce food loss, the diet was administered in non-scattering feed cups. The rats were given water through a glass tube that protruded through the cage wire. Basal diet was prepared from fine ingredients (100 g) according to **Reeves *et al*., (1993)** in the animal house of Agricultural Research Center in Ministry of Agriculture, Giza. Animal studies were approved by UResearch Animal Facility-Innstitutional Animal Care and Use Committee, Cairo University, (approval no. URAF-E-3-24).

The basal diet consisted of casein 12%, cellulose 5%, corn oil 10%, mineral mixture 4%, vitamin mixture 1%, choline chloride 0.20% and the remained amount is starch according to **AIN, (1993)** with (7.5, 10%) milk thistle powder and moringa leaves powder daily during the experimental period (6 weeks).

A total of sixty rats were housed in well-aerated cages under hygienic condition and fed on basal diet for one week for adaptation. After this week, rats were divided into twelve groups including both therapeutic and preventive groups (five rats each).

Group 1 was given a baseline diet and tap water for six weeks and will remain as the negative control group (therapeutic)**.** 25 rats will inject with CCl_4 in paraffin oil (50% v/v 4 ml/kg) to induce acute damage in liver according to **Jayasekhar *et al.*, (1997)**.This group will keep as therapeutic groups and then it will divide into five groups as follows:

Group 2 will consider as control positive (therapeutic) and will feed on basal diet, (5 weeks)

Group 3 (therapeutic) will feed on basal diet + 7.5% Milk Thistle powder replacing equivalent amount from the basal diet. (5 weeks)

Group 4(therapeutic) will feed on basal diet + 10% Milk Thistle powder replacing equivalent amount from the basal diet. (5 weeks)

Group 5(therapeutic) will feed on basal diet + 7.5% Moringa powder replacing equivalent amount from the basal diet. (5 weeks)

Group 6(therapeutic) will feed on basal diet + 10% Moringa powder replacing equivalent amount from the basal diet. (5 weeks)

Group 7 will keep as negative control group (preventive) and will feed on basal diet and tap water for 6 weeks.

Group 8 will consider as control positive (preventive) and will feed on basal diet. (5 weeks)

Group9 (preventive) will feed on basal diet + 7.5% Milk Thistle powder replacing equivalent amount from the basal diet. (5 weeks)

Group 10(preventive) will feed on basal diet +10% Milk Thistle powder replacing equivalent amount from the basal diet. (5 weeks)

Group 11 (preventive) will feed on basal diet + 7.5% Moringa powder replacing equivalent amount from the basal diet (5 weeks)

Group 12(preventive) will feed on basal diet + 10% Moringa powder replacing equivalent amount from the basal diet. (5 weeks)

Groups 8, 9, 10, 11 and 12 will inject near the end of the experiment (in the last week of the experiment) with CCl4 in paraffin oil (50% v/v 4 ml/kg) and will keep for five days after injection.

During the experimental period (6 weeks), each rat was weighed every week and feed consumption was recorded. The following formula was used to calculate the feed efficiency ratio (FER) and body weight gain in accordance with **Chapman *et al.*, (1959):**

$$(\text{BWG}) = \frac{\text{Final Weight\_ Initial Weight}}{\text{Initial Weight}}$$

$$(\text{FER}) = \frac{\text{Daily body Weight gain(g)}}{\text{Feed intake (g/d)}} x100$$

Rats were slaughtered after fasting for the entire night at the conclusion of the experiment. Following that, blood was drawn and centrifuged. Serum was separated and stored at -20°c for biochemical analysis i.e. aspartate amino transferase (AST) and alanine amino transferase (ALT) according to **Reitman & Frankel, (1957)**, serum alkaline phosphates (ALP) according to **Belfield & Goldberg, (1971)**,serum total protein(T.P) according to **Gornall *et al.*, (1949)**, According to **Doumas et al. (1971),** serum albumin (ALB) was measured by subtracting serum albumin from serum total

protein; **Young, (1996)** estimated serum total bilirubin (T.B.); **Fossati *et al*., (1980)** estimated serum uric acid; **Marsch *et al*., (1965)** estimated urea; and **Bartels & Bohmer (1971)** estimated creatinine. **NIHP (1987)** was used to calculate the total serum cholesterol. Additionally, the **Triuder, (1969)** method was used to assess the triglycerides in serum. The method described by **Friedewald *et al*., (1972)** and **Grodon & Amer. (1977)** was used to measure the high-density lipoprotein (HDL). The very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were determined according to the method of **Lee & Nieman., (1996)** as follows:

VLDL (mg/dl) = Triglycerides/5
LDL (mg/dl) = Total cholesterol - (HDL+VLDL)

A spectrophotometer (model DU 4700) was used to estimate all the parameters in serum samples, and biodiagnostic kits were used for analysis. Superoxide dismutase (SOD) was analyzed using ELISA Kit (catalog NO.CSB-E08555r) and Malondialdehyde (MDA) was analyzed using ELISA Kit (catalog NO.MBS2636626),

Histopathological Examination

Liver was separated from each rat and examined histopathologicaly according to **Bancroft & Layton, (2012).**

Statistical analysis

The mean ± SD is used to express the results. According to **McClave & Benson (1991),** one-way analysis of variance (ANOVA) was used to statistically examine the data.

RESULTS AND DISCUSSION

Chemical Composition of (Moringa & Milk Thistle) powder

Moringa& milk thistle powder were analyzed for chemical composition on the dry weight basis **Table (1)** showed that moisture , crude protein, fat content, ash, crude fiber and total carbohydrates were (6.422 , 10.896 , 6.535 , 13.052 , 8.41 and 54.685 %)on dry weight respectively of moringa and (3.630 , 4.343 , 6.060 , 23.421 , 30.66 and 31.886 %) on dry weight respectively of milk thistle **Halaby, *et al*., (2015)** found that the powdered chemical components of MO leaves The leaves powder had the highest levels of moisture, protein, ash, crude fiber, and fat (7.81, 26.31, 8.03, and 17.63%), respectively**. Giuberti *et al*., (2021)** confirmed that, moringa leaf powder has the potential to be employed as a food additive because it contains 27.4% protein, 5.6% oil, and 23.7% dietary fiber**. Amin, *et al*., (2019)** who reported that, dry milk thistle's chemical structure, Crude fiber 27.32±1.69, Protein 11.4±1.24, Fat 1.61±1.69, Carbohydrates 6.8±0.89, Moistures 19±1.24 and Ash 33.87±1.38.

Table (1): Chemical Composition of (Moringa & Milk Thistle) powder

Parameters / Samples name	Test results %					
	Moisture	**Protein**	**Fat**	**Ash**	**Crude fiber**	**Carbohydrates**
Moringa powder	6.422	10.896	6.535	13.052	8.41	54.685
Milk Thistle powder	3.630	4.343	6.060	23.421	30.66	31.886

Total antioxidants, total phenols and total flavonoids compounds of (Moringa & Milk Thistle) powder

The obtained results in **Table (2)** showed a marked significant increase in total antioxidants (87.13) DPPH and significant decrease in total antioxidants (63.59) DPPH in (MO & MT) powder (87.13) & (63.59) DPPH, respectively. Comparing total (phenols & flavonoids) powder with those parameters samples of (MO & MT) powder revealed a marked significant increase in total (phenols & flavonoids) powder (805.36) & (181.02) mg/100g as Gallic acid, respectively. The present result was in accordance with **Dharmendra Singh *et al*., (2014)** who showed that the findings indicate that the hepatoprotective and antioxidant properties of M. oleifera leaves may be linked to their ability to scavenge free radicals. This could be because the extract contains flavonoids and total phenolics, or because the purified compounds kaempferol, quercetin, and β-sitosterol were separated from the ethanol extract of M. oleifera leaves. In another study, confirmed that natural compounds called flavonoids have a number of therapeutic and medical uses. Because of their phenolic structures, several of them have antioxidant properties and prevent processes brought on by free radicals **Kidd and Head, (2005).**

Table (2): Total antioxidants, total phenols and total flavonoids compounds of (Moringa & Milk Thistle) powder

Parameters / Samples name	Active ingredients		
	Total Antioxidants (DPPH)	**Total phenols (mg/100g as galic acid)**	**Total Flavonoids (mg/100g as catachin)**
Moringa powder	63.59 ^{b} ± 3.34	805.36 ^{a} ± 17.70	807.39 ^{a} ± 6.29
Milk Thistle powder	87.13 ^{a} ± 0.77	181.02 ^{b} ± 10.42	129.33 ^{b} ± 3.14
DSL	5.50	32.92	11.27

Effect of two levels of Moringa & milk thistle powder on nutritional parameters in male rats suffering from hepatotoxicity

The **table 3** clearly shows that therapeutic positive group(2) rats have non-significant in FI, BWG and FER compared with normal control (G1) rats and G(3 ,4 , 5 , 6) rats feeding on supplemented diet with (MT & MO) powder (7.5 , 10%).On the other hand, preventive positive group(G8) and preventive groups(9,10,11&12) feeding on supplemented diet with (MT&MO) powder (7.5 , 10%)rats had no significant in FI, BWG&FER when compared with negative control group(7). **Halaby, *et al*., (2015)** reported that in comparison to a positive control group, a diet fortified with 3 percent MO (leaves or roots) can lower blood cholesterol and other lipids, as well as the risks to liver and renal function. It can also increase feed intake and body weight gain. Additionally, consistency with earlier research Rats given the MO powder leaf increased their body weight in comparison to the control group, according to studies by **Wang *et al*., (2012) and Halaby *et al*., (2015)**. Given that moringa is a nutritious food, weight gain could be the result**. Dočkalová *et al*., (2018)** found when compared to the control group, the average daily weight gain of rats fed milk thistle mixtures rose, with the group that received the 20% addition of milk thistle gaining the most weight.

Table (3): Effect of two levels of (Moringa & milk thistle) powder on nutritional parameters in male rats suffering from hepatotoxicity

Parameters / Groups		FI (g/d)	BWG (g)	FER (%)
Therapeutic	G_1	17.5 ^{b} ±0.69	0.24 ^{a} ±0.07	5.05 ^{a} ±1.25
	G_2	19.57 ^{ab} ±1.27	0.37 ^{a} ±0.06	7.38 ^{a} ±1.08
	G_3	20.83 ^{ab} ±1.10	0.55 ^{a} ±0.07	10.27 ^{a} ±1.06
	G_4	19.78 ^{ab} ±2.68	0.37 ^{a} ±0.25	7.09 ^{a} ±4.47
	G_5	18.9 ^{ab} ±1.35	0.36 ^{a} ±0.19	7.15 ^{a} ±3.34
	G_6	21.15 ^{ab} ±1.35	0.54 ^{a} ±0.22	9.97 ^{a} ±3.29
Preventive	G_7	21.63 ^{a} ±0.80	0.48 ^{a} ±0.10	9.06 ^{a} ±1.43
	G_8	18.45 ^{ab} ±0.85	0.21 ^{a} ±0.01	4.45 ^{a} ±0.12
	G_9	19.22 ^{ab} ±1.11	0.28 ^{a} ±0.06	5.87 ^{a} ±1.05
	G_{10}	20.08 ^{ab} ±0.76	0.36 ^{a} ±0.07	7.30 ^{a} ±1.16
	G_{11}	18.92 ^{ab} ±0.67	0.28 ^{a} ±0.18	5.63 ^{a} ±3.27
	G_{12}	18.98 ^{ab} ±1.21	0.26 ^{a} ±0.08	5.41 ^{a} ±1.44
	LSD	2.13	0.23	3.86

G1: negative control group, **G2**: control positive, **G3**: 7.5% Milk Thistle powder, **G4**: 10% Milk Thistle powder, **G5**: 7.5% Moringa powder and **G6**: 10% Moringa powder.
G7: negative control group, **G8**: control positive, **G9:** 7.5% Milk Thistle powder, **G10:** 10% Milk Thistle powder, **G11:** 7.5% Moringa powder and **G12**: 10% Moringa powder.

Effect of two levels of (Moringa & milk thistle) powder on liver function in both therapeutic & preventive groups of rats suffering from hepatotoxicity

The results of liver function analysis are presented in **table (4)** T.D, D.B, ALT, AST, ALP and GGT analysis values decreased significantly in groups of rats that consumed different levels of milk thistle and moringa when compared with the positive control group (therapeutic/ preventive). The group with the lowest value was G6 and G12. Then the results of the other groups that fed on milk thistle and moringa differed in decrease. Regarding the values of T.B and ID.B the groups with the lowest decrease were G5, G4, G3, G11, G10, G2, G9 and G8. The ALT analysis values for the groups with the lowest decline were G4, G11, G10, G5, G3, G9, G2 and G8. The AST analysis values for the groups with the lowest decline were G11, G4, G5, G10, G3, G9, G8 and G2. The ALP analysis values for the groups with the lowest decline were G5, G4, G11, G10, G3, G9, G2 and G8. The GGT analysis values for the groups with the lowest decline were G4, G5, G10, G11, G3, G9, G2 and G8.

As for the D.B analysis values, the groups with the lowest decrease were groups 12 and 4, followed by group 5, 6, 10, 9, 11, 3, 2 and 8 when compared with the positive control group (therapeutic/ preventive). On the other hand, T.P and ALB levels recorded a significant increase in the groups that consumed different levels of milk thistle and moringa, and the highest values were G6 and G12 in those that consumed 10% of moringa powder. These results agree with **Ibrahim Salih *et al.*, (2022)** who showed that treatment with Morenga oleifera, the liver enzymes and tissue returned to a normal state and showed non-significant ($P \leq 0.05$) differences, compared to the control group. According to the results, it can be concluded that Morenga oleifera has a great

potential to prevent and improve liver damage.

Additionally, **Elbakry, M. A. *et al*., (2019)** confirmed that treating the CCl_4 hepatonephrotoxicity Morenga oleifera significantly alleviated all altered biochemical and histological changes approaching the normal values. This protecting activity of Morenga oleifera against CCl_4 hepatonephrotoxicity may be ascribed to its high content of phenols and flavonoids, in addition to ascorbic acid and oleic acid.

Table (4): Effect of two levels of (Moringa & milk thistle) powder on liver function in both therapeutic & preventive groups of rats suffering from hepatotoxicity

Parameters / Groups		T.B (mg/dl)	D.B(mg/dl)	ID.B (g/dl)	T.P(g/dl)	ALB(g/dl)	G (g/dl)	A.G (Ratio)	ALT (U/L)	AST(U/L)	ALP (U/L)	GGT(U/L)
Therapeutic	G_1	0.45 ^{f} ±0.06	0.13 ^{c} ±0.02	0.31 ^{g} ±0.04	7.6 ^{a} ±0.38	4.36 ^{ab} ±0.16	3.24 ^{a} ±0.54	1.38 ^{a} ±0.28	22.63 ^{g} ±8.66	46.31 ^{fg} ±9.76	158.48 ^{f} ±22.67	1.81 ^{g} ±0.26
	G_2	1.03 ^{b} ±0.07	0.2 ^{ab} ±0.01	0.83 ^{bc} ±0.08	6.00 ^{e} ±0.14	3.37 ^{f} ±0.09	2.63 ^{ab} ±0.06	1.29 ^{a} ±0.03	127.28 ^{a} ±9.33	224.1 ^{a} ±27.27	466.06 ^{a} ±42.99	8.60 ^{b} ±0.55
	G_3	0.85 ^{c} ±0.05	0.17 ^{abc} ±0.01	0.68 ^{cd} ±0.05	6.38 ^{d} ±0.14	3.59 ^{e} ±0.05	2.79 ^{ab} ±0.16	1.29 ^{a} ±0.08	95.90 ^{bc} ±6.13	152.30 ^{b} ±13.78	394.88 ^{b} ±7.24	7.58 ^{cd} ±0.24
	G_4	0.73 ^{cd} ±0.08	0.14 ^{c} ±0.02	0.59 ^{de} ±0.07	6.77 ^{cd} ±0.12	3.83 ^{d} ±0.08	2.94 ^{ab} ±0.05	1.30 ^{a} ±0.02	70.8 ^{de} ±4.48	114.97 ^{cd} ±13.69	281.24 ^{cd} ±11.87	5.55 ^{e} ±0.7
	G_5	0.67 ^{de} ±0.08	0.15 ^{c} ±0.03	0.52 ^{ef} ±0.11	6.71 ^{cd} ±0.19	3.93 ^{cd} ±0.08	2.78 ^{ab} ±0.24	1.42 ^{a} ±0.15	86.35 ^{cd} ±4.51	115.91 ^{cd} ±19.85	268.48 ^{cd} ±38.13	5.76 ^{e} ±0.37
	G_6	0.57 ^{ef} ±0.07	0.15 ^{c} ±0.02	0.42 ^{fg} ±0.06	7.28 ^{ab} ±0.25	4.14 ^{bc} ±0.14	3.14 ^{a} ±0.36	1.33 ^{a} ±0.19	52.14 ^{f} ±9.34	65.18 ^{fg} ±9.24	210.16 ^{ef} ±20.48	2.72 ^{g} ±0.38
Preventive	G_7	0.55 ^{ef} ±0.07	0.13 ^{c} ±0.02	0.42 ^{fg} ±0.08	7.50 ^{a} ±0.14	4.34 ^{ab} ±0.16	3.16 ^{a} ±0.25	1.38 ^{a} ±0.16	25.86 ^{g} ±8.09	37.23 ^{g} ±8.46	172.33 ^{f} ±8.96	2.06 ^{g} ±0.55
	G_8	1.28 ^{a} ±0.03	0.21 ^{a} ±0.03	1.06 ^{a} ±0.05	5.33 ^{f} ±0.31	3.09 ^{g} ±0.12	2.24 ^{b} ±0.42	1.42 ^{a} ±0.32	129.58^{a} ±8.30	212.10 ^{a} ±17.51	485.76 ^{a} ±39.96	9.58 ^{a} ±0.62
	G_9	1.04 ^{b} ±0.06	0.17 ^{abc} ±0.01	0.87 ^{b} ±0.08	5.87 ^{e} ±0.12	3.63 ^{e} ±0.09	2.24 ^{b} ±0.18	1.63 ^{a} ±0.16	104.29^{b} ±7.90	165.53^{b} ±8.49	403.21^{b} ±18.82	8.32 ^{bc} ±0.56
	G_{10}	0.86 ^{c} ±0.04	0.16 ^{bc} ±0.03	0.7 ^{cd} ±0.06	6.73 ^{cd} ±0.15	3.95 ^{cd} ±0.07	2.78 ^{ab} ±0.16	1.42 ^{a} ±0.09	81.25 ^{d} ±3.7	137.44 ^{bc} ±11.67	327.37 ^{c} ±18.41	6.84 ^{d} ±0.24
	G_{11}	0.86 ^{c} ±0.08	0.17 ^{abc} ±0.01	0.69 ^{cd} ±0.07	6.74^{cd} ±0.08	3.97 ^{cd} ±0.13	2.77 ^{ab} ±0.21	1.44 ^{a} ±0.16	76.49 ^{d} ±5.87	94.29 ^{de} ±8.34	324.37 ^{c} ±27.09	6.87 ^{d} ±0.39
	G_{12}	0.62 ^{de} ±0.04	0.12 ^{c} ±0.01	0.5 ^{ef} ±0.03	7.06 ^{bc} ±0.13	4.46 ^{ab} ±0.08	2.61 ^{ab} ±0.11	1.71 ^{a} ±0.08	61.49 ^{ef} ±5.01	74.58 ^{ef} ±7.25	227.67^{de} ±29.88	4.07 ^{f} ±0.25
	LSD	0.10	0.03	0.11	0.33	0.18	0.45	0.28	11.89	23.85	44.65	0.76

T.B: total bilirubin - D.B: direct bilirubin – ID.B: indirect bilirubin – T.P: total protein – ALB: albumin – G: globulin – A.G: albumin. globulin - ALT: alanine transaminase test – AST: aspartate transaminase test – ALP: alkaline phosphatase – GGT: gamma-glutamyl transferase

G1: negative control group, **G2**: control positive, **G3**: 7.5% Milk Thistle powder, **G4**: 10% Milk Thistle powder, **G5**: 7.5% Moringa powder and **G6**: 10% Moringa powder.
G7: negative control group, **G8**: control positive, **G9:** 7.5% Milk Thistle powder, **G10:** 10% Milk Thistle powder, **G11:** 7.5% Moringa powder and **G12**: 10% Moringa powder.

Effect of two levels of (Moringa & milk thistle) powder on antioxidant activities of tissues in both therapeutic & preventive groups of rats suffering from hepatotoxicity

The levels of both SOD and MDA antioxidant activities of tissues of hepatotoxic rats are presented in table (5). SOD value of the therapeutic control negative group and preventive control negative group were 233.73 and 209.21 U/mg respectively. While the therapeutic control positive and preventive control positive were 19.98 and 34.51 U/mg respectively. On the other hand, the SOD value of the rats groups that fed on different levels of milk thistle and moringa leaves recorded significantly increased. The highest value of SOD was G12 and G6 followed by G4, G10, G11, G5, G3 and G9.
MDA value of the therapeutic control negative group and preventive control negative group were 0.40 and 0.89 nmol/mg respectively. While the therapeutic control positive

and preventive control positive were 16.99 and 18.65 nmol/mg respectively. Whereas, the MDA value of the rats groups that fed on different levels of milk thistle and moringa leaves recorded significantly decreased. The lowest value of SOD was G6 and G12 followed by G4, G10, G11, G5, G3 and G9.

These results agree with **Abdelazem, H., (2019)** who showed that moringa oleifera improved the activity of the enzymes (SOD, CAT, GPx and GST) associated with a decrease in the activity of MDA. Additionally, he demonstrated that moringa oleifera improved the antioxidant enzymes and the oxidative stress in rats. Similar results were obtained by **Ghaffari, A. R *et al.*, (2011**) who showed that Milk thistle stabilizes glutathione peroxidase and superoxide dismutase by raising glutathione levels.

Table (5): Effect of two levels of (Moringa&milk thistle) powder on antioxidant activities of tissues in both therapeutic & preventive groups of rats suffering from hepatotoxicity

Parameters / Groups		SOD (U/mg)	MDA (nmol/mg)
Therapeutic	G_1	233.73 ^{a} ±9.87	0.40 ^{e} ±0.05
	G_2	19.98 ^{f} ±6.90	16.99 ^{a} ±1.66
	G_3	76.11 ^{e} ±11.88	10.37 ^{b} ±1.35
	G_4	127.63 ^{d} ±7.57	4.48 ^{d} ±0.67
	G_5	106.33 ^{d} ±13.26	7.1 ^{c} ±0.45
	G_6	156.41 ^{c} ±9.67	1.88 ^{e} ±0.53
Preventive	G_7	209.21 ^{b} ±27.20	0.89 ^{e} ±0.12
	G_8	34.51 ^{f} ±8.21	18.65 ^{a} ±2.21
	G_9	75.19 ^{e} ±8.67	10.92 ^{b} ±1.46
	G_{10}	125.94 ^{d} ±19.09	6.46 ^{c} ±1.35
	G_{11}	109.18 ^{d} ±12.43	7.12 ^{c} ±1.07
	G_{12}	164.25 ^{c} ±13.61	2.78 ^{de} ±0.62
	LSD	22.81	1.94
SOD: superoxide dismutase – MDA: malodialdehyde			
G1: negative control group, **G2**: control positive, **G3**: 7.5% Milk Thistle powder, **G4**: 10% Milk Thistle powder, **G5**: 7.5% Moringa powder and **G6**: 10% Moringa powder. **G7**: negative control group, **G8**: control positive, **G9:** 7.5% Milk Thistle powder, **G10:** 10% Milk Thistle powder, **G11:** 7.5% Moringa powder and **G12**: 10% Moringa powder.			

Effect of two levels of (Moringa & milk thistle) powder on serum kidney in both therapeutic & preventive groups of rats suffering from hepatotoxicity

Table(6) showed that untreated therapeutic, preventive rats(G2, G8) have significant increase in serum levels of creatinine, urea and uric acid compared with those of normal control therapeutic, preventive rats(G1,G7). In contrast, rats feeding on supplemented diet with (MT 7.5 , 10% & MO 7.5 , 10%) therapeutic , preventive groups had significantly decreased serum levels of creatinine, urea & uric acid comparing with those of positive control groups (untreated, therapeutic & preventive) G (2,8) and significant decrease in serum (Cr) and (UA) preventive (G12),which nearly returned toward the normal levels preventive (G7). The findings are consistent with

those of **Nouri and Heidarian, (2019),** who found that milk thistle herbs caused rats' serum uric acid levels to gradually drop in all treated groups. Additionally, **Amin *et al*. (2019)** found that, in comparison to the control positive group, creatinine, serum urea, and uric acid significantly decreased in all acute renal failure groups administered with varying concentrations of dried milk thistle (20% and 40%). Affirmed by **Halaby *et al*., (2015)** suggest that a 3% high concentration of MO-fortified food, either in the form of roots or leaves was safe and enhances renal function. Findings indicated that the kind and concentration of MO in either the roots or the leaves caused a progressive decrease in creatinine levels, the ratios reached to 1.06 and 1.19 for supplementation diet with roots or leaves powder at 1.5%, while the ratios reached to 0.72 and 0.70 compared with positive control group 1.60. According to our findings, MO at high concentrations enhanced nutritional value and had the greatest impact on kidney function **Buraimoh, (2011).** Our results agree with **Dharmendra Singh *et al*., (2014)**stated that *M. oleifera* leaf extract was found to significantly ($p \leq 0.001$) lower serum levels of SGOT, SGPT, GGT, LDH, ALP, ACP, and total bilirubin in CCl4-induced rats, while also significantly increasing total protein and albumin at all three dose levels (Group III–V) in comparison to CCl4-treated Group II. The most effective dose level of M. oleifera was 400 mg/kg. Variations in rats' serum alkaline phosphatase (ALP) activity following different administrations of M. oleifera leaf extract. Also **Amin, A. F. *et al*., (2019)** confirmed it may be beneficial to include dried milk thistle in the diet to counteract the negative effects of glycerol or CCl_4 on kidney, liver, and lipid profiles**. Mayer *et al*., (2005)** discovered that the group that took milk thistle MT extract had lower mean BUN and creatinine levels.

Table (6): Effect of two levels of (Moringa&milk thistle) powder on serum kidney in both therapeutic & preventive groups of rats suffering from hepatotoxicity

Parameters / Groups		Creatinine (mg/dl)	Urea (mg/dl)	Uric Acid (mg/dl)
Therapeutic	G_1	0.55 ^{e} ±0.07	21.85 ^{d} ±2.50	2.95 ^{de} ±0.10
	G_2	1.17 ^{a} ±0.05	45.92 ^{a} ±2.48	5.02 ^{a} ±0.18
	G_3	0.99 ^{b} ±0.06	38.66 ^{b} ±1.62	4.34 ^{b} ±0.16
	G_4	0.8 ^{cd} ±0.05	30.79 ^{c} ±1.31	3.73 ^{c} ±0.15
	G_5	0.68 ^{de} ±0.09	31.44 ^{c} ±2.59	3.80 ^{c} ±0.24
	G_6	0.57 ^{e} ±0.09	22.57 ^{d} ±2.84	3.05 ^{de} ±0.21
Preventive	G_7	0.55 ^{e} ±0.07	18.60 ^{d} ±1.04	2.72 ^{e} ±0.44
	G_8	0.95 ^{bc} ±0.10	43.31 ^{ab} ±3.34	4.59 ^{b} ±0.24
	G_9	0.83 ^{cd} ±0.06	38.78 ^{b} ±1.79	3.87 ^{c} ±0.13
	G_{10}	0.85 ^{bc} ±0.08	30.82 ^{c} ±1.32	3.29 ^{d} ±0.22
	G_{11}	0.69 ^{de} ±0.06	40.78 ^{b}±2.32	3.95 ^{c} ±0.30
	G_{12}	0.55 ^{e} ±0.06	28.54 ^{c} ±2.75	3.1 ^{de} ± 0.14
	LSD	0.12	3.82	0.38

G1: negative control group, **G2**: control positive, **G3**: 7.5% Milk Thistle powder, **G4**: 10% Milk Thistle powder, **G5**: 7.5% Moringa powder and **G6**: 10% Moringa powder.
G7: negative control group, **G8**: control positive, **G9:** 7.5% Milk Thistle powder, **G10:** 10% Milk Thistle powder, **G11:** 7.5% Moringa powder and **G12**: 10% Moringa powder.

Effect of two levels of (Moringa & mil thistle) powder on lipid profile in both therapeutic & preventive groups of rats suffering from hepatotoxicity

The results of lipid profile analysis are shown in table (7). The values of T.C, T.G, LDL and VLDL were significantly decreased in the rats that consumed different levels of milk thistle and moringa when compared with the positive control group (therapeutic/ preventive). The lowest declines were recorded in groups 6 and 12, which were fed 10% moringa powder. After that, the results varied in the decrease; as the T.C values for the lower groups were G5, G4, G9, G11, G10, G3, G8 and G2. The groups which had the lowest T.G values and VLDL were G9, G10, G5, G11, G3, G4, G8 and G2. The groups which had the lowest LDL values were G5, G4, G9, G11, G8, G10, G3 and G2. While HDL levels increased significantly in the groups that fed on different levels of milk thistle and moringa when compared with the positive control group (therapeutic/ preventive). The groups with the highest increase were group G6 and G12, which were fed 10% moringa powder. These results are accordance with data by **Chen *et al.*, (2020)** who reported that TC, HDL-C, LDL-C, and T.G levels were reduced by extract from the leaves of Moringa oleifera.

Additionally, **Dubey *et al.*, (2013)** confirmed that the Moringa oleifera plant significantly decreased cholesterol levels and atherosclerotic formation to approximately 50% and 86%, respectively. They also demonstrated that rats' cholesterol levels decreased when they were given powdered Moringa oleifera leaves orally. It was determined that Moringa oleifera leaves have definite hypocholestemic action and that using them for this purpose has a sound pharmacological foundation.

Table (7): Effect of two levels of (Moringa&milk thistle) powder on lipid profile in both therapeutic & preventive groups of rats suffering from hepatotoxicity

Parameters / Groups		T.C (mg/dl)	T.G (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Therapeutic	G_1	103.2 ^{f} ±13.45	91.86 ^{d} ±11.58	48.41 ^{abc} ±1.63	36.42 ^{f} ±10.41	18.37 ^{d} ±2.32
	G_2	233.35 ^{a} ±10.70	163.02 ^{a} ±8.20	43.24 ^{bc} ±2.52	157.5 ^{a} ±10.27	32.60 ^{a} ±1.64
	G_3	193.45^{b} ±7.65	137.79 ^{b} ±3.11	46.75 ^{abc} ±4.75	119.14 ^{b} ±8.18	27.55 ^{b} ±0.62
	G_4	165.52 ^{cd} ±7.02	138.71 ^{b} ±10.38	45.70 ^{abc} ±5.07	92.08 ^{bc} ±11.96	27.74 ^{b} ±2.07
	G_5	153.11 ^{d} ±9.13	121.02 ^{bc} ±12.73	48.88 ^{abc}±1.52	80.03 ^{cd} ±12.44	24.20 ^{bc} ±2.55
	G_6	126.15 ^{e} ±9.67	95.64 ^{cd} ±6.68	50.77 ^{a} ±2.37	56.25 ^{ef} ±9.21	19.13 ^{cd} ±1.33
Preventive	G_7	95.69 ^{f} ±6.28	72.97 ^{d} ±3.41	45.91 ^{abc} ±2.47	35.19 ^{f} ±8.86	14.59 ^{d} ±0.68
	G_8	194.54 ^{b} ±7.85	143.11 ^{b} ±19.37	49.28 ^{abc} ±1.33	116.64 ^{b} ±6.28	28.62 ^{b} ±3.88
	G_9	167.21 ^{cd} ±9.09	118.42 ^{bc} ±7.81	44.83 ^{abc} ±0.80	98.70 ^{bc} ±6.82	23.68 ^{bc} ±1.56
	G_{10}	183.27 ^{bc} ±16.40	119.64 ^{bc} ±18.44	42.10 ^{c} ±2.05	117.25 ^{b} ±15.21	23.92 ^{bc} ±3.69
	G_{11}	176.66 ^{bcd} ±11.68	136.71 ^{b}±7.39	49.98 ^{ab} ±1.15	99.34^{bc} ±13.38	27.34 ^{b} ±1.48
	G_{12}	128.0 ^{e} ±11.80	97.05 ^{cd} ±8.28	46.53 ^{abc} ±0.77	62.06 ^{de} ±16.87	19.41 ^{cd} ±1.66
	LSD	18.75	18.44	4.35	18.98	3.69

T.C: total cholesterol – T.G: triglycerides – HDL: high density lipoprotein – LDL: low density lipoprotein – VLDL: very low density lipoprotein

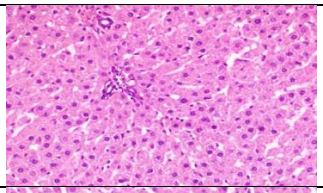
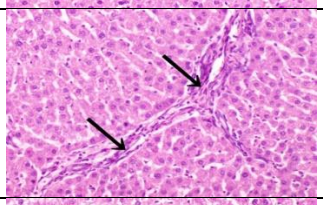
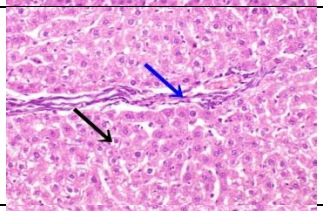
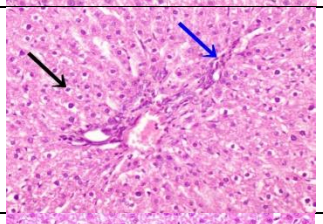
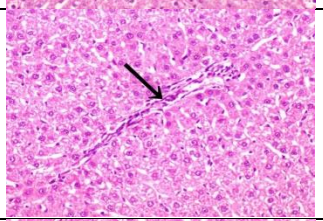
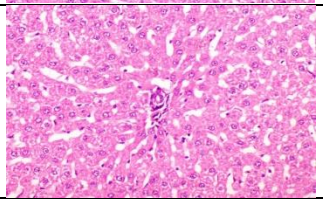
G1: negative control group, **G2**: control positive, **G3**: 7.5% Milk Thistle powder, **G4**: 10% Milk Thistle powder, **G5**: 7.5% Moringa powder and **G6**: 10% Moringa powder.
G7: negative control group, **G8**: control positive, **G9:** 7.5% Milk Thistle powder, **G10:** 10% Milk Thistle powder, **G11:** 7.5% Moringa powder and **G12**: 10% Moringa powder.

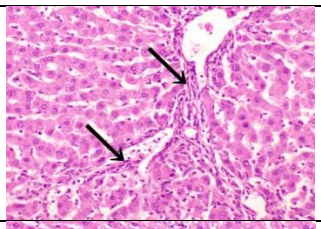
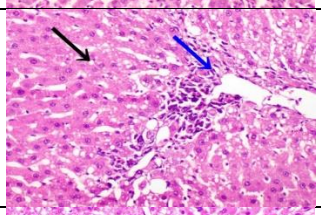
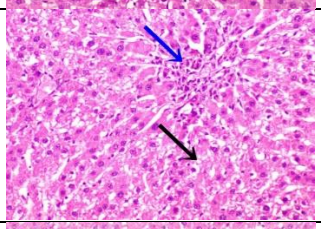
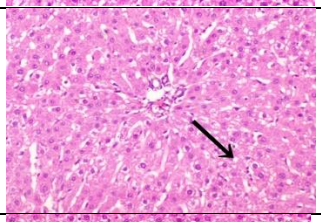
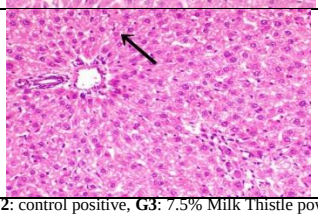
Histopathological Examinations

Histopathological examination of liver:

Light microscopy examination of liver sections of rats from G_1 (normal control) (therapeutic) revealed the normal histoarchitecture of hepatic parenchyma (Figure 1). In adverse, liver of rats from G_2(untreated group) (therapeutic) exhibited histopathological lesions characterized by coagulative necrosis of centrilobular hepatocytes, hepatocellular apoptosis, massive fibroblasts proliferation in the portal triad and extend to encircle the hepatocytes (Figure 2) as well as portal infiltration with inflammatory cells. Meanwhile, hepatic sections of rats from G_3(7.5%MT powder) (therapeutic) showed mild hepatocellular vacuolar degeneration and moderate strands of fibroblasts in the portal triad (Figure 3). Furthermore, liver of rats from G_4(10%MT powder) (therapeutic) demonstrated mild hepatocellular vacuolar degeneration and fine strands of fibroblasts in the portal triad (Figure 4). Moreover, liver of rats from G_5(7.5%MO powder) (therapeutic) revealed mild hepatocellular vacuolar degeneration and moderate strands of fibroblasts in the portal triad (Figure 5) as well as oval cells proliferation. On the other hand, liver sections from G_6(10%MO powder) (therapeutic) showed Kupffer cells activation, mild vacuolization of some hepatocytes and fine strands of fibroblasts in between the hepatocytes (Figure 6). Otherwise, liver of rats from G_7(normal control) (protective) exhibited normal histoarchitecture of hepatic tissue (Figure 7). In contrariwise, liver of rats from G_8(untreated group) (protective) showed severe changes as coagulative necrosis of centrilobular hepatocytes, hepatocellular apoptosis associated with inflammatory cells infiltration, massive fibroblasts proliferation in the portal triad and extend to encircle the hepatocytes (Figure 8) as well as portal infiltration with inflammatory cells. However, liver of rats from G_9 (7.5%MT powder) (protective) showed vacuolar degeneration of hepatocytes, hepatocellular apoptosis associated with fine strands of fibroblasts proliferation (Figure 9). Furthermore, sections from G_{10}(10%MT powder) (protective) described slight vacuolar degeneration of some hepatocytes and hepatocellular apoptosis (Figure 10). Moreover, liver of rats from groups (11 & 12) (7.5% & 10% MO powder) (protective) exhibited normal portal area, slight Kupffer cells proliferation and slight vacuolar degeneration of some hepatocytes (Figures 11 & 12).Our results agree with **Dharmendra Singh *et al.*, (2014)**who cleared that the histomorphological image of liver sections treated with 400 mg/kg of M. oleifera extract and CCl4-induction revealed a lobular pattern that was largely normal and free of degenerative alterations. Also **Dočkalová *et al.*, 2018)** said that the hepatoprotective activity of silymarin may have contributed to the reduced incidence of liver steatosis in the animals fed pressed milk thistle components. The results from the histological perspective showed that the 10% group was the most suitable amount of milk thistle pressed components. **Al-dabbagh *et al*., (2022)**reported that in addition to being a preventative step against the risk of diabetes and its complications, milk thistle is efficient in repairing damaged hepatic cells.Another study by **Janice Post-White *et al*., (2007)**revealed that Silymarin is a strong antioxidant that promotes detoxification pathways, repairs liver tissue, and stabilizes cell membranes.Also reported that a natural supplement called milk thistle (Silybum marianum) is used to treat biliary and liver conditions. The main ingredient in silymarin, a blend of flavonoid complexes, shields kidney and liver cells from the harmful effects of medications like chemotherapy.The restoration of these markers to

nearly normal levels indicates that the administration of silymarin (SIL) for 30 days effectively reduced these DEN-induced abnormalities in the liver tissue. This might be because SIL has a protective impact on the hepatocellular membranes, preserving their integrity and halting more cellular damage. These results are consistent with those of **El-Samaligy *et al.*, (2006),** who found that silymarin protected rats from CCl4-induced hepatotoxicity in a comparable way.

Organ / Groups		Photomicrograph of liver	Discussion
Therapeutic	G1		**Fig (1): Showing the normal histoarchitecture of hepatic parenchyma (H & E X 200).**
	G2		**Fig (2): Showing massive fibroblasts proliferation in the portal triad and extend to encircle the hepatocytes (black arrows) (H & E X 200).**
	G3		**Fig (3): Showing mild hepatocellular vacuolar degeneration (black arrow) and moderate strands of fibroblasts in the portal triad (blue arrows) (H & E X 200).**
	G4		**Fig (4): Showing mild hepatocellular vacuolar degeneration (black arrow) and fine strands of fibroblasts in the portal triad (blue arrows) (H & E X 200).**
	G5		**Fig (5): Showing mild hepatocellular vacuolar degeneration (black arrow) and moderate strands of fibroblasts in the portal triad (blue arrows) (H & E X 200).**
	G6		**Fig (6): Showing fine strands of fibroblasts in between the hepatocytes (black arrows) (H & E X 200).**
Preventive	G7		**Fig (7): Showing the normal histoarchitecture of hepatic parenchyma (H & E X 200).**

	G8		**Fig (8): Showing massive fibroblasts proliferation in the portal triad and extend to encircle the hepatocytes (black arrows) (H & E X 200).**
	G9		**Fig (9): Showing vacuolar degeneration of hepatocytes (black arrow), hepatocellular apoptosis associated with fine strands of fibroblasts proliferation (blue arrow) (H & E X 200).**
	G10		**Fig (10): Showing slight vacuolar degeneration of some hepatocytes (black arrow) and hepatocellular apoptosis (blue arrow) (H & E X 200).**
	G11		**Fig (11): Showing slight vacuolar degeneration of some hepatocytes (black arrow) (H & E X 200).**
	G12		**Fig (12): Showing slight vacuolar degeneration of some hepatocytes (black arrow) (H & E X 200).**

G1: negative control group, **G2**: control positive, **G3**: 7.5% Milk Thistle powder, **G4**: 10% Milk Thistle powder, **G5**: 7.5% Moringa powder and **G6**: 10% Moringa powder.

G7: negative control group, **G8**: control positive, **G9:** 7.5% Milk Thistle powder, **G10:** 10% Milk Thistle powder, **G11:** 7.5% Moringa powder and **G12**: 10% Moringa powder.

CONCLUSION

Milk thistle and moringa are natural herbs that show promising effects in treating liver problems. It contains joint benefits of protecting liver cells from damage, reducing hepatic inflammation, reducing liver toxicity and lowering cholesterol levels. They also had a therapeutic and preventive effect on the damage caused by carbon tetrachloride and improved the biochemical parameters of the liver, kidneys and lipid profile.

REFERENCES

Abdelazem, H. (2019). Effect of Moringa oleifera on antioxidant enzymes and oxidative stress induced by aluminium exposure in male albino rat testes. *International Journal of Cancer and Biomedical Research*, *3*(3), 34-41.

AIN (American Institute of Nutrition). (1993). Purified diet for laboratory Rodent; Final report. J. Nutrition. 123:1939-195.Compacted Berth. *Journal of Essential Oil Research*. 8(6): 657-664.

Amin, A. F., Mosa, Z. M., Nagib, E. W., & Abdelrahman, A. R. (2019). Effect of feeding artichoke and milk thistle on rats treated with CCl4 and glycerol/saline solution. *African Journal of Biological Sciences*, 15(1), 1-12..

AOAC, (2005). Association of official analytical chemists, 18th edition. Washington, DC: Association of Official Analytical Chemists.

Al-dabbagh, B. M. A., Abed, M. N., Mahmood, N. M., Jasim AAFMH, A. M., & Thanoon, I. A. J. (2022). Anti-inflammatory, antioxidant and hepatoprotective potential of milk thistle in albino rats. Latin American journal of pharmacy, 14(9), 1832-1841.

Bartels, H. & Bohmer, M. (1971). Creatinine standard and measurement of serum creatinine with picric acid. *Clin. Chem*.; Acta, 32:81.

Belfield, A. & Goldberg, D.M. (1971). Alkaline Phosphatase Colorimetric Method". *Journal of Enzyme*, (12): 561.

Bijak, M. (2017): Silybin, a major bioactive component of milk thistle (*Silybum marianum L. Gaernt*.) chemistry, bioavailability, and metabo- lism. Molecules, 22, 1942.

Buraimoh, A. A.(2011). Hepatoprotective effect of ethanolic leave extract of Moringa oleifera on the histology of paracetamol induced liver damage in wistar rats.Int.J.Anim.vet.Adv.,3:10-13.

Bancroft, J. D., & Layton, C. (2012). The hematoxylins and eosin. Bancroft's theory and practice of histological techniques, 7, 173-186.

Chapman, D.G.; Castilla,R. & Champell, J.A.(1959).Evaluation of protein efficiency ratio , *Canadian Journal of Biochemistry and Physiology*,37:679-686.- **Charoensin, S.(2014).** Antioxidant and anticancer activities of Moringa oleifera leaves. J. Med. Plants Res. 8, 318–325.

Chen, G. L., Xu, Y. B., Wu, J. L., Li, N., & Guo, M. Q. (2020). Hypoglycemic and hypolipidemic effects of Moringa oleifera leaves and their functional chemical constituents. *Food chemistry*, *333*, 127478.

Choksi ,S.;Patel ,S.S and Saluja, A.K .(2000): Silymarin—a promising herbal hepatoprotective drug.Ind Drugs 37: 566–569.

Dharmendra Singh.; Priya ,V. A.; Ved, P. A and Radhey, S. G.(2014). Evaluation of Antioxidant and Hepatoprotective Activities of Moringa oleifera Lam. Leaves in Carbon Tetrachloride-Intoxicated Rats. Journal Antioxidants.3, 569-591.

Dočkalová, H., Horký, P., Zeman, L., & Skládanka, J. (2018). Influence of milk thistle pressed parts on rats liver histology. Potravinárstvo**.**

Doumas, B. T., Watson, W. A., & Biggs, H. G. (1971). Albumin standards and the measurement of serum albumin with bromcresol green. *Clinica chimica acta*, 31(1), 87-96.

Dubey, D. K., Dora, J., Kumar, A., & Gulsan, R. K. (2013). A multipurpose tree-Moringa oleifera. *International journal of pharmaceutical and chemical sciences*, *2*(1), 415-423.

Elbakry, M. A., El Rabey, H. A., Elremaly, W., Sakran, M. I., & Almutairi, F. M. (2019). The methanolic extract of Moringa oleifera attenuates CCl4 induced hepatonephro toxicity in the male rat. *Biomed Res*, *30*(1), 23-31.

Elghandour,M.; Maggiolino,A.; Vazquez-Mendoza,P. (2023). "*Moringa oleifera* as a natural alternative for the control of gastrointestinal parasites in equines: a review," Plants, vol. 12(9), p. 1921.

El-Samaligy, M.S.;Afifi, N.N and Mahmoud, E.A. (2006). Evaluation of hybrid liposomes-encapsulated silymarin regarding physical stability and in vivo performance. Int J Pharmacy .319: 121–129.

Ezejindu, D.; Udemezue,O and Chinweife,K.(2013). Hepatoprtective effects of Moringa oleifera extract on liver of wistar rats. Vol. 3, No.5.

FAO .(1982). Food Composition Tables for the Near East, F.A.O., Food and Nutrition Paper, 26

Fozia Farooq.; Meenu Rai.; Avinash Tiwari.;Abdul Arif, K and Shaila Farooq.(2012). Medicinal properties of Moringa oleifera: An overview of promising healer. Journal of Medicinal Plants Research Vol. 6(27), pp. 4368-4374.

Friedewald, W.T; Levy, R. I. and Fredrickson, D. S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 18: 499-502.

Ghaffari, A. R., Noshad, H., Ostadi, A., Ghojazadeh, M., & Asadi, P. (2011). The effects of milk thistle on hepatic fibrosis due to methotrexate in rat. *Hepatitis monthly*, *11*(6), 464.

Giuberti ,G.; Rocchetti ,G.; Montesano, D and Lucini, L. (2021). The potential of Moringa oleifera in food formulation: a promising source of functional compounds with healthpromoting properties. Current Opinion in Food Science.42: 257–269.

Gornall,A.C.; Bardawill,C.J & David,M.M.(1949).Determination of serum protein. *Journal of Biological Chemistry*, 177:751.

Grodon, T. and Amer, M. (1977): Determination of HDL, J. American of Medicine. 62(5):707-714.

Guan, Y. S. and He, Q. (2013). A current update on the rule of alternative and complementary medicine in the treatment of liver diseases. Evidence-Based Complementary and Alternative Medicine, Article ID 321234:1-10.s.

Halaby, M. S., El-Din, M. M., & Emara, N. E. (2015). Influence of Moringa oleifera on non-alcoholic fatty liver in adult albino rats. Middle East. J. Appl. Sci, 5(4), 902-912.

Halaby, M.; Elmetwaly,E and Omar,A(2013). Effect of Moringa oleifera on serum lipids and kidney function of hyperlipidemic rats. Egyptian J. Appl. Sci. Res., 9(8): 5189-5198.

Ibrahim Salih, A., Mohammed Saleh, H., Sulaiman Khalaf, A., & Sabbar Ayed, H. (2022). Effect of moringa oleifera leaves against hepatotoxicity induced by bisphenol A. *Archives of Razi Institute*, *77*(3), 1083-1089.

Janice Post,W.; Faan, R.N .; Elena, J. L and Kara, M. K.(2007). Advances in the Use of Milk Thistle (Silybum marianum). Integrative Cancer Therapies. 6(2); pp. 104-109.

Jayasekhar, P., Mohanan, P. V., & Rathinam, K. (1997). Hepatoprotective activity of ethyl acetate extract of Acacia catechu. *Indian J Pharmacol*, 29(6), 426.

John, B. I.;Sulaiman, C. T.; George, S and Reddy, V. R. (2014). Total phenolics and flavonoids in selected medicinal plants from Kerala. International Journal of Pharmacy and Pharmaceutical Sciences. 6(1): 406-408.

Kidd, P and Head, K.(2005). A review of the bioavailability and clinical efficacy of milk thistle phytosome: a silybin-phosphatidylcholine complex (Siliphos). Altern Med Rev . 10: 193-203.

Lee, R. and D. Nieman, (1996). Nutritional Assessment.2nd Ed., Mosby, Times. Mirror Co. St. Louis, Missouri.

Lina, N.A .; Teti Estiasih.; Wenny, B. S and Alfi Khatib.(2023). Potential of moringa (Moringa oleifera) leaf powder for functional food ingredients: A review. Czech Journal of Food Sciences, 41, (1): 8–20.

Loguercio, C.; Federico ,A.; Trappoliere ,M.; Tuccillo, C.; de Sio, I and Di Leva ,A.(2007). The effect of a silybin-vitamin e-phospholipid complex on nonalcoholic fatty liver disease: a pilot study. Dig Dis Sci. 52(9):2387-95.

Luka, C.D.; Tijjani,H.; Joel,E.B.; Ezejiofor,U.L and Onwukike,P.(2013). Hypoglycaemic Properties of Aqueous Extracts of Anacardium occidentale, Moringa oleifera, Vernonia amygdalina and Helianthus annuus: A Comparative Study on Some Biochemical Parameters in Diabetic Rats, International Journal of Pharmaceutical Science Invention, PP.16-22.

Marsh, W. H., Fingerhut, B., & Miller, H. (1965). Automated and manual direct methods for the determination of blood urea. *Clinical chemistry*, 11(6), 624-627.

Mayer ,K.E.;Myers, R.P and Lee, S.S.(2005). Silymarin treatment of viral hepatitis: a systematic review. J Viral Hepat. 12(6):559-67.

McClave, J.T., and Benson, P. G.(1991). Statistical for business and economics. Max Well Macmillan International editions. Dellen Publishing Co. USA.272-295.

Milić, N.; Milošević, N.; Suvajdžić, L.;Žarkov, M and Abenavoli ,L .(2013). New therapeutic potentials of milk thistle (Silybum marianum). Nat Prod Commun 8(12): 1801-1810.

Mohammadi ,S.; Kianbakht, S.;Rezazadeh, S.; Ziaee, M and Fallah Huseini H. (2020). Clinical efficacy of Silybum marianum seed extract in treatment of type 2 diabetes mellitus and non-alcoholic fatty liver disease: a narrative review. Journal of Medicinal Plants 19(73): 12-26.

NIHP (1987): Detection, Evaluation and treatment of high cholesterol in adults, National Institute of Health Publication. 88:292.

Nouri, A. and Heidarian, E. (2019). Nephroprotective effect of silymarin against diclofenac-induced renal damage and oxidative stress in male rats. Journal of Herb: Med. Pharmacology, 8(2), 146–152.

Okwari, O.; Dasofunjo ,K.; Asuk,A.; Alagwu,E and Mokwe,C. (2013). Anti-Hypercholesterolemic and Hepatoprotective effect of Aqueous Leaf Extract of Moringa oleifera in Rats fed with Thermoxidized Palm Oil Diet. Journal of Pharmacy and Biological Sciences (IOSR-JPBS) e-ISSN: 2278-3008, p-ISSN: 2319 - 7676. 8 (2): 57-62.

Oliveira, J.; Silveira,S.; Vasconcelos,K.; Cavada,B and Moreira,R.(1999). Compositional and nutritional attributes of seeds from the multiple purpose tree Moringa oleifera Lamarck. J Sci Food Agric. 79(6):815-820.

Pepping J.(1999). Milk thistle: Silybum marianum. Am J Health Syst Pharm. 56(12):1195-7.

Reeves, P. G.; Nielsen, F. H. & Fahmy, G. C. (1993). AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *Journal of Nutrition*; 123(11):1939-1951.

Reitman, S. & Frankel, S. (1957). Determination of glutamate pyruvate transferase. *American Journal of Clinical Pathology*, 28:56.

Selvakumar, D and Natarajan, P .(2008). Hepato-protective activity of Moringa oleifera Lam. leaves in carbon tetrachloride induced hepatotoxicity in albino rats. Pharmacogn. Mag. 4:97-98

Sreelatha ,S and Padma ,P.R. (2010). Protective mechanisms of Moringa oleifera against CCl(4)-induced oxidative stress in precision-cut liver slices. Forsch Komplementmed. 17, 189-194.

Su, M. S., & Chien, P. J. (2007). Antioxidant activity, anthocyanins, and phenolics of rabbiteye blueberry (Vaccinium ashei) fluid products as affected by fermentation. *Food chemistry*, 104(1), 182-187.

Taj Mohammadi ,A.; Razavi, B. M and Hosseinzadeh, H. (2018). Silybum marianum (milk thistle) and its main constituent, silymarin, as a potential therapeutic plant in metabolic syndrome: A review. Phytother Res 32(10): 1933-1949.

Triuder, p. (1969): En jymatic colorimetrie determination of triglycoride by GPO-PAP Method, Ann.Clin. Biochem.. 6: 24-27

Wang, L.; Sun,J.; Oida,Y.; Wang,X and Xingrong,J.(2012). Protective effect of polyphenols Extract of Adlay (Coxi lachrymal Jobi, L.Var.ma-yuen-stapf). On hypercholestremia induced oxidative stress in Ruts molecules .17:8886-8897.

Young, D.S.(1996).Effects of drugs on clinical Lab Tests,4th ed AACC press.

Yousef, M.I.; Omar, S.A.; El-Guendi, M.I and Abdelmegid, L.A. (2010). Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. Food Chem Toxicol.48, 3246-3261.