



Potential Effect Of Milk Thistle (*Silybum Marianum*) On Liver Disorders Induced By Carbon Tetrachloride

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Abstract

This study was conducted to investigate the effect of milk thistle on rats with induced by carbon tetrachloride Thirty mature albino rats weighting 150-160 gm B.wt. each were used, and divided into 6 equal groups, one was kept as a control (- ve) group, while the other groups were injected s/c by (0.2 ml/kg) carbon tetrachloride twice a week for Two weeks. The used plants were given as a percent of 0.5% ,1%,1.5% and 2% from the basel diet., Serum liver function (AST, ALT and ALP), total cholesterol and triglycerides, lipoproteins (HDL-c, LDL-c, VLDL-c) were determined kidney functions (urea, creatinine, uric acid). The obtained results concluding that feeding with milk thistle herb improved, liver functions, kidney functions and lipid profile.

Key words: Milk Thistle, Liver Function, Lipid Profile, Kidney Functions .

Introduction

Liver is the largest solid organ, the largest gland and one of the most vital organs that functions as a center for metabolism of nutrients and excretion of waste metabolites. Its primary function is to control the flow and safety of substances absorbed from the digestive system before distribution of these substances to the systemic circulatory system (Allen , 2002). A total loss of liver function could leads to death within

minutes, demonstrating the liver's great importance (**Ozougwu and Eyo .,2014**).

There are many types of liver disease that can be caused by a virus, damage from drugs or chemicals, obesity, diabetes or an attack from your own immune system (**Talal et al., 2013**).

Nature has been a source of medicinal agents since the beginning of time. Herbal medicine is still the most common source for primary health care of about 65-80% of the world's population, mainly in developing countries, because of better cultural acceptability, better compatibility with the human body and fewer side effects. Leaves, flowers, stems, roots, seeds, fruit and bark can all be constituents of herbal medicines. The medicinal values of these plants lie in their phytochemical components which produce definite physiological actions on the human body. The most important of these components are alkaloids, tannins, flavonoid and phenolic compounds (**Shariff, 2001**).

Silybum marianum (L.) commonly known as milk thistle (MT), is an annual / biennial plant of the Asteraceae family, native of Mediterranean area and now growing and cultivated worldwide (**Abenavoli et al., 2010 and Bijak, 2017**).

MT has been used for centuries in medicine, mainly to treat kidney, spleen, liver, and gallbladder disease .The Roman naturalist and natural philosopher Pliny the Elder (23–79 AD) wrote that mixing the juice of this plant with honey was indicated to “carry off the bile.” The Greek physician, pharmacologist, and botanist Dioscorides (i.e., the author of *De Materia Medica*) recommended it as tea against serpent bites (**Abenavoli et al., 2018**).

MT was also popular in the German medical tradition and several scientists, including Johannes Gottfried Rademacher (1772–1850), recommended it to treat liver ailments. In the USA, the popularity of MT derives from its use as a part of the naturopathic medical tradition of the Native Americans as well as of the Eclectic movement, a group of practitioners that recommended MT for varicose veins, menstrual problems, and congestion of the spleen, kidney, and liver in the first half of 19th century. Actually, MT is among the top-selling herbal dietary supplements in the USA with retail sales amounting to 2.6 million dollars in the mainstream multioutlet channel in 2015 (**Andrew and Izzo, 2017**).

Aim of study:

This study aims to identify the antioxidant activities of milk thistle on liver disorders with induced by carbon tetrachloride, and study effects on liver functions, kidney functions lipid profile.

Materials and methods

.Milk thistle were obtained from local market in shebin El-kom

Carbon tetrachloride (CCl₄):

Carbon tetrachloride was obtained from Elgomhoria Company for med-preparations chemicals and Medical Equipments, Cairo-Egypt as 10% liquid solution. It was dispensed in white plastic bottles each containing one liter as a toxic chemical material for liver poisoning according to **Passmore and Eastwood, (1986)** in the same time it is mixed with 10% paraffin oil which obtained from the pharmacy for dilution during the induction.

Rats

Thirty adult male albino rats, weighting 150-155g. from Medical Insects Research Institute, Doki, Cairo, were used in this study.

Methods:

Preparation of materials:

M.T was grinded in to soft powder by using Electric grinder and kept in dusky stoppered glass bottles in a cool and dry location till use according to **Russo, (2001)** who reported that al herbs and plants are pest kept in a cool, dry, and dark location to reduce oxidation of their contents.

Biological Experiments:

Basal diet:

The basic diet prepared according to the following formula as mentioned by (**AIN, 1993**) as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride(0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). Used vitamin mixture component was that recommended by **Campbell, (1963)** while the salt mixture used was formulated according to **Hegsted *et al* , (1941)**.

Preparation of liver impaired rats:

Liver impaired was induced in normal healthy male albino rats by subcutaneous injection of CCl₄ (0.2 mg/kg body weight) for Two weeks according to method described by **Passmore and Eastwood, (1986)**

Experimental Design:

The experiment was done in the Faculty of Sciences, Menoufia University, Shebin El-kom. Rats were housed in wire cages in a room temperature and kept under normal healthy conditions.

Rats were divided into the following groups:

Group 1: feed basal diet, as a negative control group (5 rats).

In this group rats were kept on standard diet and tap water.

Group 2: induced group (25 rats)

In this group, rats were induced by 0.2 mg/kg body weight by Carbon Tetrachloride for two weeks to induce liver impaired. This group

was subdivided into 5 subgroups (each 5 rats) to feed on the experimental diets for 4 weeks according to the following:

Sub group (2): positive control group (untreated group)

Sub group (3): treated with 0.5% of milk thistle.

Sub group (4): treated with 1% of milk thistle.

Sub group (5): treated with 1.5% of milk thistle.

Sub group (6): treated with 2% of milk thistle.

Blood Samples

Blood samples were collected after 12 hours fasting at the end of the experiment using the abdominal aorta in which the rats were scarified under ether anesthetized. Blood samples were received in to clean dry centerfuge tubes and left to clot at room temperature, then centerfuged for 10 minutes at 3000 rpm to separate the serum. Serum was carefully aspirate, transferred in clean cuvet tubes, and stored frozen at -20°C for analysis. All serum samples were analyzed for determination the following parameters

• **Determination of Liver Functions:**

Serum Alkaline Phosphates (Alp): was determination of according to **Belfield and Goldberg , (1971)**.

Serum glutamic oxaloacetic transaminases (AST) and glutamic pyruvic transaminase (ALT). were measured according to method described by **Yound, (1975)** and **Tietz, (1976)**.

■ **Estimation of serum lipid:**

Triglycerides was carried out according to **Fassati and precipe, (1982)**. Total cholesterol was determined according to **Allen, (1974)**.

HDL-cholesterol: by the same method used for total cholesterol, according to **Lopez, (1977)**.

VLDL -c and LDL-c were calculated according to the method of **Lee and Nieman, (1996)** as follows:

VLDL (mg/dl) = Triglycerdes/5

LDL (mg/dl) = (Total cholesterol – HDL-c) – VLDL-c

Determination of Renal Functions:

Urea was determined according to the enzymatic method described by of **Patton and Crouch, (1977)** Creatinine was determined according to kinetic method of **Henry, (1974)**, and Uric acid was carried at according to method of **Schultz, (1984)**.

Statically analysis:

The data were statistically analyzed using a computerized costat program by one way ANOVA. The results are presented as mean± SD. Differences between treatments at ($P \leq 0.05$) were considered significant. **Snedecor and Cochran , (1979)**.

Results and Discussion

AST, ALT and ALP of hepatic rats and consumed milk thistle

Table (1) illustrate the mean value of AST, ALT and ALP hepatic rats fed on various diets. It could be noticed that AST of control (-) group was lower than control (+) group by the ratio of 45.24 %. All hepatic rats fed on different diets revealed significant decreases in mean values as compared to control (+) group. The values were 203.5 ± 24.88 , 235 ± 24.88 , 198 ± 24.88 and 204.5 ± 24.88 for milk thistle 0.5%, 1%, 1.5% and 2% milk thistle respectively . respectively. Groups 3,4,5 and 6 showed nonsignificant differences between them. The best AST was recorded for group 5 and 6 (hepatic rats fed on milk thistle 1.5%, and 2%). As , It could be observed that the mean value of ALT of control (+) group was higher than control (-) group, being 93 ± 14.6 and 70.5 ± 14.6 (by a percent of 24,19%) All treated groups showed significant decreases of ALT with relative percentages (42.3%, 54.83% , 57.35% and 50.53%) for groups 3,4,5 and 6 respectively . compared to positive group . In the same table ,it could noticed that an increase in the ALP level of the positive group ,then a significant decrease occurred through the treated groups compared to positive group .The best was recorded for group 5,6 (hepatic rats fed on milk thistle 1.5% and 2%).

these results in agreement with **Kumar and Khanna .,(2018) and Amin et al., (2019)** , they injected that all groups treated with CCl4 or glycerol/ saline solution and administrated with different levels of dried milk thistle (20% -40%) had a significant decrease in their liver function, compared with the positive control group.

Table (1) : Effect of milk thistle on Serum AST, ALT and ALP activities (u\l) of hepatic rats .

Groups	AST (U\l)	ALY(U\l)	ALP (U\l)
	Mean±SD	Mean±SD	Mean±SD
Control (-)	$210^b \pm 24.88$	$70.5^b \pm 14.61$	$147.33^c \pm 14.60$
Control (+)	$383.5^a \pm 24.88$	$93^a \pm 14.61$	$320.33^a \pm 14.60$
Milk thistle 0.5%	$203.5^{bc} \pm 24.88$	$53.66^c \pm 14.61$	$232.66^c \pm 14.60$
Milk thistle 1%	$235.5^{bc} \pm 24.88$	$42^c \pm 14.61$	$276.5^b \pm 14.60$
Milk thistle 1.5%	$198^c \pm 24.88$	$39.66^c \pm 14.61$	$202.5^d \pm 14.60$
Milk thistle 2%	$204.5^{bc} \pm 24.88$	$46^c \pm 14.61$	$203.33^d \pm 14.60$
L.S.D (p≤0.05)	24.88	14.61	14.60

Means in the same column with different litters are significantly ($p \leq 0.05$) different .,

Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-c) , Low Density Lipoprotein Cholesterol (LDLc) and Very Low Density Lipoproteins Cholesterol (VLDLc) of hepatic rats treated with milk thistle .

Table (2) Illustrate the mean value of (TC) of hepatic rats fed on different diets. It could be observed that the mean value of TC of positive group 115.63 was higher than negative group 89.33 ± 2.96 , showing significant when milk thistle was added to the diet of rats , significant decrease occurred compared to positive group, especially group 3 (0.5% milk thistle) which recorded the lowest level of TC. For TG , It could be noticed that the mean value of control (+) group was 204.4 ± 1.84 higher than control (-) group 132.3 ± 1.84 . All treated group by M.T show a significant decreases in TG levels compared to positive control group .The best result was recorded for group 5 and 6 (hepatic rats fed on milk thistle 1.5%, and 2%) . The mean value of (HDL.c) of positive group 24 ± 2.81 was lower than negative group 30 ± 2.81 showing hepatic rats fed on different diets. It could be observed that the mean value of (HDLc) of control (+) group was lower than control (-) group 30 ± 2.81 , significant when milk thistle was added to the diet of rats , significant increase occurred compared to positive group, especially, group 6 (2% milk thistle) which recorded the highest level of HDL. for LDL.C It could be observed that the mean value of control (+) group was 139.5 ± 2.32 higher than control (-) group 75.8 ± 2.32 , All treated groups by M.T show a significant decreases in LDL levels compared to positive control group. The best result was recorded for group 6 (hepatic rats fed on milk thistle 2%) . For VLDL.C It could be observed that the mean value of VLDLc of positive group 40.8 ± 0.36 was higher than negative group 26.4 ± 0.36 , showing significant difference when milk thistle was added to the diet of rats , significant decrease occurred compared to positive group, especially group 5 and 6 (1.5% milk thistle and 2%) which recorded the lowest level of This study agree with (Dabbour *et al.*, 2014) and (Amin *et al.*, 2019) reported that the ratio of Milk Thistle Seed oil was the main factors affecting the lipid profile found that the plasma levels of TC, LDL, VLDL, liver cholesterol and liver TG in rats treated with silymarin decreased compared to hyper lipidemic rats and negative control.

Table(2). Effect of milk thistle on Total cholesterol ,Triglycerides , (HDL.c) , (LDLc) and (VLDLc) of hepatic rats.

Groups	T.C (mg/dl)	T.G (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control (-)	89.3 ^c ±2.69	132.3 ^e ±1.84	30 ^a ±2.81	75.8 ^d ±2.32	26.4 ^e ±0.36
Control (+)	115.3 ^a ±2.69	204.4 ^a ±1.84	24 ^c ±2.81	139.5 ^a ±2.32	40.8 ^a ±0.36
Milk thistle 0.5%	95 ^d ±2.69	146.2 ^b ±1.84	29 ^{ab} ±2.81	87.9 ^b ±2.32	29.2 ^b ±0.36
Milk thistle 1%	99.43 ^c ±2.69	142.5 ^c ±1.84	26 ^{bc} ±2.81	88 ^b ±2.32	28.5 ^d ±0.36
Milk thistle 1.5%	98 ^c ±2.69	140.2 ^d ±1.84	29 ^{ab} ±2.81	83.1 ^c ±2.32	28.02 ^d ±0.36
Milk thistle 2%	107.3 ^b ±2.69	140.5 ^d ±1.84	30.6 ^a ±2.81	81.7 ^c ±2.32	28.1 ^d ±0.36
L.S.D (p<0.05)	2.69	1.84	2.81	2.32	0.36

Means in the same column with different letters are significantly (p ≤ 0.05). different.

Urea, Creatinin and Uric acid of hepatic rats and consumed milk thistle.

Table (3) :Illustrate the mean value of urea of hepatic rats fed on various diets. It could be noticed that the mean value of urea of control (+) group was higher than control (-) group, being 46.4 ±1.70 and 39.9±1.70 the percentage of decrease was 14 % .All treated rats decrease (12.06%, 13.6%,15.08% and 19.8% for groups 3,4,5 and 6) when compared to positive group 5 and 6 (milk thistle 1.5% and 2%) recorded the best value of Urea level. . As for creatinin it could be observed that the mean value of creatinin of control (+) group was higher than control (-) group,1.06±0.14 and 0.75±0.14 when added different levels of milk thistle , showed significant decrease for groups 4,5 and 6 (1%, 1.5% and 2%) The best level was recorded for group 4,6. As for Uric Acid levels recorded clear decrease compared with positive control group. The lower value was 1.73± 0.31 for group 5 (1.5% milk thistle). All The percent of decreases were 42.2%, 37.1%, 67.3%and-52.8%for groups 3,4,5 and 6 respectively . This result agrees with **Nouri and Heidarian,(2019)** they reported rats showed a progressive decrease serum uric acid of all treated groups as affected by milk thistle herbs. Also, **Amin et al.,(2019)** observed that all acute renal failure groups administrated with different levels of dried milk thistle (20% and 40%) had significant decrease in creatinine, serum urea and uric acid compared with the control positive group .

Table (3) Urea , Creatinin and Uric acid of hepatic rats and consumed milk thistle

Groups	Urea (mg\dl)	Creatinin (mg\dl)	Uric acid (mg\dl)
	SD±Mean	SD±Mean	SD±Mean
Control (-)	39.9 ^b ±1.70	0.75 ^b ±0.14	2.99 ^b ±0.31
Control (+)	46.4 ^a ±1.70	1.06 ^a ±0.14	5.3 ^a ±0.31
Milk thistle 0.5%	40.8 ^b ±1.70	0.92 ^{ab} ±0.14	3.06 ^b ±0.31
Milk thistle 1%	40.07 ^b ±1.70	0.78 ^b ±0.14	3.33 ^b ±0.31
Milk thistle 1.5%	39.4 ^b ±1.70	0.84 ^b ±0.14	1.73 ^d ±0.31
Milk thistle 2%	37.2 ^c ±1.70	0.81 ^b ±0.14	2.5 ^c ±0.31
L.S.D (p≤0.05)	1.70	0.14	0.31

Means in the same column with different litters are significantly (p ≤ 0.05) different.

Conclusion

In conclusion, milk thistle was shown to be an effective therapeutic agent that could alleviate liver disorder through the stimulation of liver regeneration and inhibition of hepatic. This may point out the hepatoprotective effect of milk thistle improved, liver functions, kidney functions and lipid profile.

References

- Abenavoli, L.; Capasso, R.; Milic, N. and Capasso, F. (2010):** Milk thistle in liver diseases: Past, present, future. *Phytotherapy Research*, 24, 1423–1432.
- Abenavoli, L.; Izzo, A. A.; Milić, N.; Cicala, C.; Santini, A. and Capasso, R. (2018):** Milk thistle (*Silybum marianum*): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. *Phytotherapy Research*, . 32, 2202–2213.
- AIN, (American Institute of Nutrition), (1993):** Purified diet for laboratory Rodent, Final report. *J. Nutrition*. 123:1939-1951. and *O. compactum Benth. J. essential oil Res.* 8 (6): 657-664.
- Allen, S.E.(2002):** The liver: Anatomy, Physiology, Disease and Treatment. North Eastern University Press, USA.

- Allen, C.C. (1974):** Cholesterol enzymatic colorimetric method. J. of Clin. Chem., (20): 470.
- Amin, A. ; Mosa, Z.; Nagib, E. and Abdelrahman, A. (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.
- Andrew, R. and Izzo, A. A. (2017):** Principles of pharmacological research of nutraceuticals. British Journal of Pharmacology, 174, 1177–1194
- Belfied, A. and Goldberg, D.M. (1971):** Alkaline phosphatase colorimetric method. J. of Enzyme, (12): 561.
- Bijak, M. (2017):** Silybin, a major bioactive component of milk thistle (*Silybum marianum* L. Gaernt.) chemistry, bioavailability, and metabolism. Molecules, 22, 1942.
- Campbell, J.A. (1963):** Methodology of protein evaluation. RAG. Nutr. Document R. 10 Led. 37: June Meeting. New York.
- Dabbour, I. R. ; Ahmad .M. N.; Takruri. H. R., and Al- Ismail K. M. (2014):** Effect of chemical properties of milk thistle seed oil on serum lipid profile and antioxidants capacity in rats fed high cholesterol and cholesterol free diets. Pakistan J. Nutr. 13(10): 600-609
- Fassati, P. and Prencipe, L. (1982):** Triglyceride enzymatic colorimetric method. J. of Clin. Chem., (28): 2077.
- Hegsted ,D. ; Mills ,R.; Elvehjemand, C. and Hazt , M. (1941)** Choline in the nutrition Biol.Chem,138:459.
- Henry, R.J. (1974):** Clinical Chemistry Principal and Techniques. 2nd ed. Harper and Publisher. New York.
- Kumar, S. and Khanna, R.K. (2018):** Beneficial effects of *Silybum marianum* seed extract against hepatic fibrosis induced by carbon tetrachloride in rats . BMJ journal 67, (2):AB.CO 265.
- Lee, R. and Nieman, D. (1996):** National Assessment. 2nd Ed., Mosby, Missouri, USA. Liver Diseases .Dig. Dis. Sci.; 50 (10): 1807-1812.
- Lopez, M.F. (1977):** HDL- cholesterol colorimetric method. J. of Clin. Chem., (230): 282
- 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.

- Ozougwu J.C, and Eyo J.E (2014):** Hepatoprotective effects of *Allium cepa* extracts on paracetamol-induced liver damage in rat. *African Journal of Biotechnology*, 13(26): 2679 -2688.
- Passmore, R. and Eastwood, M.A. (1986):** "Human Nutrition and Dietetics". Eight editions. Longman Group UK LTD. Churchill Livingstone.
- Patton. C.J. and Croush, S.R. (1977):** Enzymatic Determination of Urea. *J. Anal. Chem.* 49: 464-469.
- Russo, E. (2001):** "Hand book of Psychotropic Herbs A scientific Analysis of Herbal Remedies for Psychiatric conditions". The Howrth Herbal press. Inc.
- Schultz, A.(1984):** Uric Kaplan A. *Clin Chem.* Mosby Co. St. Louis Toronto. Princeton; 1261-1266 and 41
- Sendcor,G and Cocharn ,W.(1979):**"Statistical method 6th ed".Iowa State Collage ,Pub , U.S.A:841.
- Shariff, Z.U. (2001):** Modern Herbal Therapy for Common Ailments. Nature Pharmacy Series Vol.1, Spectrum Books Ltd., Ibadan, Nigeria in Association with Safari Books (Export) Ltd. UK, pp. 9-84.
- Talal, A. M.; David, S.; Barnes, M.; William, D.; Carey, M.; Srinivasan, D. M.; Milan, D. M.; Kyrsten, F. M. and Winans, M. (2013):** Understanding Liver Disease Treatment and Options. NIH Publication, No. 14-113, 1-16.
- Tietz, N. W. (1976):** Fundamentals of Clinical Chemistry. Philadelphia. B.W. Standers, P.243.
- Yound, D.S. (1975):** Determination of GOT. *Clin. Chem.*, 22 (5): 21.

التأثير المحتمل لشوكة الجمل على اضطرابات الكبد المستحثة بواسطة رابع كلوريد الكربون

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المستخلص العربي

تم اجراء الدراسة الحالية لمعرفة التأثيرات المحتملة لشوكة الجمل على الخلل الفسيولوجي الحادث في الكبد. تم استخدام 30 فأر أبيض بالغ يتراوح اوزانهم بين 150-160 جم وتم تقسيمهم الى 6 مجموعات متساوية احدهم كمجموعة ضابطة سالبة أما المجموعات الأخرى فتم احداث إصابة بالكبد عن طريق الحقن بواسطة رابع كلوريد الكربون بمعدل 0.2 ملجم/كجم من وزن الجسم مرتين في الاسبوع و لمدة اسبوعين وتم اضافة شوك الجمل للوجبة الأساسية على هيئة مطحون بنسبة 0.5% و1% و1.5% و2%. وتم قياس مستوى انزيمات الكبد (AST,ALT,ALP) والكوليستيرول الكلى والجليسيريدات الثلاثية والليبيروتينات (HDL-c-LDL-c, VLDL-c) وظائف الكلى (اليوريا, الكرياتينين, حمض اليوريك) .. وقد أظهرت نتائج هذه الدراسة أن تناول شوك الجمل تعمل على تحسن في وظائف الكبد والكلى ودهون الدم.

الكلمات المفتاحية: شوك الجمل - وظائف الكبد-وظائف الكلى-ودهن الدم.