www.fagr.bu.edu.eg

Annals of Agricultural Science, Moshtohor (ASSJM)
https://assjm.journals.ekb.eg/

© 2023, Faculty of Agriculture, Benha University, Egypt. ISSN:1110-0419

Original Article Vol. 62(4) (2024), 41 – 50 DOI: 10.21608/assjm.2025.330994.1322

Evaluation of the Effect of Milk Thistle Extract on Gamma Irradiated Rats

Zeinab H.H.Gad[1,2], Enas Mahmoud Mekawi [1],Mohamed H.M. Abd el-Megid[2] and Salah M.M. Saad[1]

1- Biochemistry Department, Faculty of agriculture, Benha University

2- Natural Products Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt

* **Corresponding Author: dr.zenab2017@gmail.com**

Abstract

The milk thistle (*Silybum marianum*) is a good source of phenolic and flavonoid compounds that act as natural antioxidants. The present study was planned to investigate the protective role of milk thistle (MT) against γ-radiation (six gray) induced hepatotoxicity and renal toxicity. Liver markers, kidney function, lipid profile, and malondialdehyde (MDA) as an oxidative stress marker, and antioxidant enzyme (CAT, SOD, and GSH) were measured after radiation. The obtained results showed that oral administration of milk thistle extract (200mg /kg B.w) orally daily for four weeks caused significantly decrements in the activities of transaminase enzymes in the serum of rats, and significantly decrements in the total lipids ,triglyceride and total cholesterol levels in serum of irradiated rats. Also caused improvement in kidney function, and significant increments in the antioxidant enzyme (SOD and CAT) and GSH concentration. Significant in decrements total free radical and MDA in blood and Liver tissues have happened with irradiated groups .This study suggests that (MT) has an antioxidant effect to radio protective effect, this may be due to its advantage of using (MT) as a source of natural antioxidants against gamma radiation

Keywords: gamma irradiation , milk thistle , liver ,kidneys , irradiated rats

Introduction

Ionizing radiation produces various free radicals inside the body causing oxidative stress when exposed to it. It leads to damage to the vital compounds in different tissues inside the body ***(Xhuti et al., 2023)***.Common sources of radiation exposure include radiotherapy, medical diagnosis, and various imaging protocols. The generation of reactive oxygen species (ROS) by γ-irradiation leads to oxidative stress that damages multiple organs ***(Kim et al. 2017).***

Milk thistle (*Silybum marianum.)* is considered as flowering plant that belongs to the Asteraceae family. It grows mainly in the Mediterranean regions of Europe. However, it can also be found in some parts of Asia, Africa, America ,and Australia. ***(Porwal et al .,2019).*** The milk thistle is a rich source of phytochemicals compounds with multiple biological interests. The silymarin is main bioactive compound and it is composed of a mixture of flavonolignans: silybin A, silybin B, silydianin, silychristin, isosilybin A, and isosilybin B,***(Federico and Dallio2017)***. Silymarin has been shown to prevent damage to the liver through several mechanisms: including; inhibition of lipid peroxidation, anti-inflammation **(Daryoush et al., 2018).** Administration of dietary antioxidants has been suggested to protect against liver tissue damage induced by radiation exposure. Silymarin is a polyphenolic plant flavonoid derived from Silymarin marianum that has hepatoprotective and anticarcinogenic effects. ***(El-Shennawy et al ., 2016).*** Silymarin has a potential attenuating effect on acute kidney injury due to its antioxidant, anti-inflammatory and antiapoptotic actions ***(Naseer et al ., 2023).***, inhibits the production of pro-inflammatory cytokines generated by leukocytes (***(Costa et al., 2016***). The aim of the present study is a trial to investigates the effect of milk thistle plant extract (200mg /kg B.w) as a natural antioxidant against gamma irradiated rats (six gray doses)

MATERIALS AND METHODS

2.1. Materials

Milk thistle flowers used in the present study were obtained from local market as dried herbal plant, Egypt, Cairo

All chemicals used in these experiments were provided by Sigma and Aldrich chemical company of high quality and purity

2. 2. Experimental Animals

Twentyeight male albino rats weighing between 150 and 170 g were obtained from the Faculty of Pharmacy Cairo University Cairo, Egypt ,the animals were kept for two weeks for the adaptation in an animal house (Nuclear Research center

Atomic Energy, Authority) and at standard condition of food and water for two weeks ***(El-Hadary and Ramadan 2019)***

2. 3.Method

2. 3.1 Extraction of plants

The flower of plant were cleaned by hand picking from foreign materials then the plants were crowded and shaken over night with 5 volume of 95% ethanol and filtered through Whatman NO. 1 paper for twice repetition. The filtrates were subjected to vacuum distillation at 35C° to remove the solvent using rotary evaporator and antioxidant extract according to ***(Spingo and Faveri 2007***)

2. 3. 2 Determination of total phenolic compound

Phenolic compound were determined according to ***(Singleton and Rossi 1965).*** One-milliliter extract was added to 5 mL distilled water then 1 mL of Folin-Ciocalteu reagent and 1 mL of sodium carbonate (20%) was added. After standing for 30 min in dark under ambient room temperature, the absorbance was measured using spectrophotometer at 765 nm UV/Vis spectrophotometer (SM1600 UV–vis Spectrophotometers, Azzota, USA). Phenolic in the extracts was expressed as gallic acid equivalent.

2. 3. 3 Determination of total flavonoids

Flavonoids were determined by the aluminum chloride colorimetric method and expressed as quercetin equivalents (***Meda et al., 2005***). One milliliter of diluted extract was mixed with 1 mL of 2% (w/v) methanolic AlCl3, 100 ML 1 M CH3 COOK, and 2.8 mL distilled water were added and then kept for 30 min at room temperature. Absorbance was measured at 765 nm using UV–vis spectrophotometer (SM1600 UV–vis Spectrophotometers, Azzota, USA).

2. 3. 4 Determination of total antioxidant capacity

2, 2-diphenylpicrylhydrazyl (DPPH·) antiradical test was performed according to the ***(Blois, 1958)*** with minor modification. DPPH· stock solution was prepared (0.004% w/v) in methanol. One mg sample was dissolved in methanol and 0.1 mL diluted sample was mixed with 3.9 mL stock solution with vigorous shaking. The solution was kept in dark for 30 min then the absorbance was measured at 517 nm using UV–Vis spectrophotometer (SM1600 UV–vis Spectrophotometers, Azzota, USA) against the absorbance of the DPPH. Ascorbic acid was used as a standard reference and % of DPPH· de-coloration was calculated as follows:

% of DPPH· de- coloration=100× (*A*2 −*A*1⁄*A*2)

Where *A*1 is the control absorbance and *A*2 is the sample absorbance.

2. 3. 5 Identification of phenols and flavonoids by HPLC

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 0.9 mL/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A) ; 15-16 min (82% A) and 16-20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µL for each of the sample solutions. The column temperature was maintained at 40 °C.

2. 3. 6. Irradiation

Whole-body γ-irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt, using a 137Cs Gamma Cell-40 biological irradiator. Animals were exposed to fractionated doses of γ-radiation, six gray ***(El-Shennawy et al., 2016)*** dose of rat 1Gy/3min at the time of irradiation

2. 3. 7 Experimental Designs

A total of (28) male albino rats weighting 150-170 g were used, seven rats in each group

Group 1: (Negative group) un irradiated rats were fed with basal diet for four weeks.

Group2: (positive control) irradiated rats (six gray) were fed with basal diet for four weeks.

Group3: un irradiated rats were fed on basal diet and received milk thistle extract 200mg/kg B.w. orally daily for four weeks

Group4: Irradiated rats were fed on basal diet and received milk thistle extract 200mg/kg B.w Orally daily for four weeks

2. 2. 8 Blood collection

Samples of blood at two days and at the end of the experiment (four weeks) were taken using heparinized capillary tubes. Each sample was divided into two tubes. The first tube contained EDTA for the measurement of total free radicals. The second tubes were allowed to clot. Serum was separated by centrifuging for 15 min at 3,000 rpm.

2. 2. 9 Liver tissue collection

Samples of liver tissue at two days and at the end of the experiment (four weeks) were Anesthesia rats using diethyl ether were collected fresh, for measuring glutathione, catalase superoxide dismutase, malondialdehyde and total free radicals

2. 2. 10. Blood analysis

The biochemical analysis included liver enzyme activities of alanine transaminase (ALT) and aspartate transaminase (AST), were determined according to ***(Reitman and Frankel 1957)*** method, kidney functions i.e. creatinine was determined according to ***Bartles etal., 1972*** and urea and was determined according to ***(Fawcett and Soctt 1960)*** , Lipid profile [TL (total lipids), TG (triglycerides),and TC (total cholesterol), were determined according to**(*Zollner and Kirsch 1962*), (*Fossati and Prencipe. 1982*)** and **(*Richmond 1973*). and (*Allain et al., 1974*).** respectively**,** Free radical

capacity in blood and tissue of liver (EPR Spectrometer) were determined according to***(Gohn, 1986) and(Heckly , 1975).*** Malondialdehyde (MDA) , catalase activity (CAT) , superoxide dismutase activity (SOD), and glutathione concentration in serum and liver tissue were determined according to ***(Satoh, 1978) and (Ohkawa et al., 1979)***, ***(Aebi, 1984)***, ***(Nishikimi et al., 1972)*** and **(Beutler etal., 1963)** respectively

2. 2. 11 Statistical analyses

All results were presented as mean ± *SD* of the mean and statistical analyses were carried out with ANOVA using SPSS 13.0 software ***(SAS, 1996).***

RESULTS A ND D ISCUSSION

3.1. Total phenolic (TP) and Total flavonoids (TF) content in ethanoic extracts of plants

Many fruits, vegetables, and herbs contain a great variety of phytochemicals such as phenolic compounds and flavonoids, Phenolic and flavonoid compounds which play important roles in scavenging free radicals as act reactive species oxygen, reactive spices nitrogen, and nitric oxide. The phytochemicals are considered as a rich source of natural which have various clinical properties such as anti-atherosclerotic, anti-inflammatory, antitumor, and antiviral. ***(Saboon et al., 2019).***Therefor the total phenolic compound and total flavonoid compound were determined in the ethanolic extract of milk thistle. The obtained results are shown in Table *(1).*These results indicate that the total phenolic content percentages equaled (1.53%). while the total flavonoids were (1.11) %in milk thistle these. The obtained results are in agreement with that obtained by (***Tupe et al., 2013***). and slightly than (***Ismaili et al., 2016***). While higher than (***Mhamdi et al., 2016***). and (***javeed ., 2022).*** The different results depend on the different parts of plants which were extracted

Antioxidant capacity

Antioxidants play an important role in biological cells by neutralizing free radicals, which can negatively affect on living organisms. Antioxidant activities of milk thistle (MT) has strong antioxidant capacity as shown in Table (1) ,the milk thistle has *(*65.05 ± 3.7*)* % in ethanoic extracts. These results were strongly correlated with their polyphenolic and flavonoid contents that had high scavenging free radicals, because these compounds reduce and discolor DPPH through their ability to donate hydrogen. The accomplished results are in agreement with the results obtained by ***(Ines et al., 2020).***

Table 1. Total phenolic, total flavonoid content, and antioxidant activity of milk thistle extracts

Phytochemical	Milk thistle
Total phenolics%	1.53± 02
Total flavonoids%	1.11± 02
Antioxidant activity%	65.05 ± 3.7

3.2 Identification of phenolic and flavonoid Compounds in milk thistle extract by HPLC chromatography

Table **(**2**)** show that there were 14 phenolic compounds in milk thistle ethanolic extract, were identified. The main characterized compounds and their contents (µg/mL) were chlorogenic acid (14.53) followed by naringenin (9.06), gallic acid (6.69) , ellagic acid (3.64), coumaric acid (3.49), syringic acid (3.43), apigenin (3.30), caffeic acid. (2.15), querectin. (2.03), methyl gallate (0 .72), vanillin (0.61), ferulic acid (0.38), daidzein (0.37), cinnamic acid (0.12), reported by **Janmejai and Sanjay., 2009**. ***,Korany et al., 2013. , XIU-LAN et al 2009.*** And ***Lucie etal., 2020***).

Table 2. Phenolic and flavonoid compounds (µg/mL) in milk thistle extract analyzed by HPLC chromatography

Compounds	Conc. (µg/mL)
Gallic acid	6.69
Chlorogenic acid	14.53
Catechin	0.00
Methyl gallate	0.72
Caffeic acid	2.15
Syringic acid	3.43
Pyro catechol	0.00
Rutin	0.00
Ellagic acid	3.64
Coumaric acid	3.49
Vanillin	0.61

Ferulic acid	0.38
Naringenin	9.06
Daidzein	0.37
Querectin	2.03
Cinnamic acid	0.12
Apigenin	3.30
Kaempferol	0.00
Hesperetin	0.00

3.3The Effect of the milk thistle extract on liver function of irradiated rats

Data presented in Table 3 show a significant increment in liver enzymes (AST and ALT) levels in the gamma irradiated group treated with six gray after two days and four week after radiation comparing with normal control .The observed increment in serum AST and ALT in γ-irradiated animals in this study could potentially be attributed to the profound physiological impact of irradiation, resulting from either direct interaction between cellular membranes and γ-rays or through the action of radiation-produced free radicals. Following radiation therapy, alterations in the enzymatic activities may result from the release of enzymes from radiosensitive tissues or from modifications in their synthesis, and they may also be connected to the widespread destruction of the liver parenchyma (***El – Shahat et al., 2022)***.

Production of free radicals this in turn increases the cytoplasmic membrane permeability to organic substances and causes leakage of cytosolic enzymes such as ASTand ALT ***(Weiss and Lander 2003).***

Milk thistle significantly restored elevated liver enzyme levels. These observations may be due to the

Presence of natural liver-protective bioactive compounds in milk thistle extract, which can lessen liver damage brought on by free radicals. This suggests that these substances are beneficial. Repair and replenish liver cells, enhance liver health and function, shield the liver from injury, and stop additional harm to the liver parenchyma ***(Vahid et al 2023).***

Table 3. Effect of the milk thistle extract on liver enzyme level in serum of irradiated rats

Treatment / Groups	AST (U/ml)		ALT (U/ml)	
	After2days	After four weeks	After2days	After four weeks
-ve control	139.79±3.8	137.82±2.1^{b}	239.55±6.9	238.04±4.03^{b}
+ve control	220.88±2.8^{a}	471.90±7.5^{a}	285.67±5.5^{a}	298.48±4.5^{a}
MT	136.19±3.9^{b}	136.27±4.5^{b}	227.95±7.5^{b}	232.07±8.6^{b}
IR+MT	220.99±5.0^{a}	141.98±2.2^{b}	282.55±3.2^{a}	251.67±4.1^{b}

a Significant change with –ve control <0.05 ,b significant change with +ve control <0.05 at the same time,(MT) milk thistle(,IR) gamma irradiation

3.4 The Effect of the milk thistle extract on kidney function of irradiated rats

The kidneys play a crucial role in controlling the body's fluids, electrolytes, and acid-base metabolism. Kidneys excrete waste metabolites; modulate the blood pressure ***(Klaus et al 2021)***.

A significant increment was noted in the serum urea and creatinine, levels in irradiated groups after two days and four weeks compared with the normal control (Table 4). These results are in agreement with that reported by ***El-Shahat et al., (2022)*** who noted that the result of this study showed that whole body γ-irradiation of rats has induced a significant increment in the concentration of serum urea and creatinine. The high level of urea might be attributed to addition-induced amino acids catabolism. The increased protein catabolic rate in irradiated rats is accompanied by a decrease in liver total proteins and an increment in the content of non-protein nitrogen of both liver and serum as well as increased levels of serum amino acids and ammonia which depends mainly upon the protein destruction after irradiation. Therefore, the orally administration of milk thistle extract has important role to protect the kidneys damage against gamma irradiation thes may be due to significant reducing the level of creatinine and urea in serum of irradiated rats .the accomplished reported agree with that reported by ***Abdel-Mobdy etal .,2021., Elkady et al., 2023., and Naseer et al 2023***

Table 4. Effect of the milk thistle extract on creatinine and urea levels in serum of irradiated rats

Treatment / Groups	Creatinine mg/dL		Urea mg/dL	
	Two days	Four weeks	Two days	Four weeks
-ve control	0.287±0.006	0.285±0.005[b]	32.565±0.09	32.757±0.52[b]
+ve control	0.772±0.10[a]	0.975±0.015[a]	60.301±0.79[a]	70.475±1.1[a]
MT	0.272±0.008[b]	0.272±0.010[b]	30.033±0.88[b]	30.047±1.01[b]
IR+MT	0.772±0.018[a]	0.305±0.005[b]	60.327±1.3[a]	34.482±0.55[b]

a Significant change with –ve control <0.05 ,b significant change with +ve control <0.05at the same time, ,(MT) milk thistle(,IR) gamma irradiation

3.5 The Effect of the milk thistle extract on lipid profile in serum of irradiated rats

In irradiated group levels of total cholesterol, triglyceride and total lipids are significantly increments than in the normal group. Ionizing radiation caused oxidative stress, which could affect on hepatic lipid metabolism and serum lipoprotein levels. Radiation exposure is linked to the development of oxidative stress and increased levels of lipid fractions (***Hanan et al., 2022)***. The profile of serum lipids in normal, irradiation, and received milk thistle extract rats has shown in Table 5. Serum TL, TC, and TG, levels were significantly increased in irradiated rats after two days and four weeks comparing with normal control. These results agree with the reported by ***Hamza et al., (2013),*** noticed that radiation caused oxidative stress and free radical generation, which changed lipid metabolism and can be a major reason of hormonal imbalance. This imbalance induces hyperlipidemia, hypercholesterolemia and hypertriglyceridemia, moreover, A decrement in the activity of the enzyme cholesterol 7ahydroxylase and an increase in the activity of 3-hydroxyl methyl glutaryl COA (HMG-CoA) reductase may be associated with hypercholesterolemia, as may the liver's increased capacity for cholesterol biosynthesis. ***(Moussa et al.,2015)*** as well as It is possible that inhibition of lipoprotein lipase activity causes a reduction in adipose cell uptake of triglycerides, leading to hypertriglyceridemia. ***(El-Dein et al., 2016).***

Such observed data in Table 5 revealed a noticeable protective property for the milk thistle extract against gamma radiation damage for serum of total lipids ,triglyceride, and total cholesterol levels , therefore ,it could be concluded that the oral administration of the tested plant from milk thistle has important role to decreases the total lipids triglyceride and total cholesterol levels level in serum against gamma and protect tissues from oxidative stress from gamma irradiation these results are consistent with that reported by ***Hanan et al., 2022., Saara et al., 2020. and Ola et al., 2020.***

Table 5. Effect of the milk thistle extract on lipids profile in the serum of irradiated rats

Treatment / Groups	Total lipids mg/dL		Triglycerides mg/dL		Total cholesterol mg/dL	
	Two days	Four weeks	Two days	Four weeks	Two days	Four weeks
-ve control	992.27±27.4	991.13±15.8[b]	68.65±0.98	71.19±1.13[b]	99.70±1.42	101.62±1.62[b]
+ve control	1334.05±17.4[a]	1440.26±22.9[a]	91.25±1.18[a]	95.19±1.35[a]	121.31±1.57[a]	131.18±2.03[a]
MT	930.72±27.1[b]	931.18±31.2[b]	69.76±2.01[b]	69.80±2.34[b]	99.08±2.83[b]	99.14±3.33[b]
IR+MT	1334.69±29.6[a]	973.82±15.5[b]	91.30±2.07[a]	73.02 1.16[b]	121.37±2.82[a]	103.45±1.65[b]

a Significant change with –ve control <0.05 ,b significant change with +ve control <0.05 at the same time, ,(MT) milk thistle(,IR) gamma irradiation

3.6 oxidative stress marker

3.6 .1 the effect of milk thistle extract on free radicals in irradiated rats

The data shown in Table (6) observe significantly increment in total free radicals in blood and liver tissue of rats after two days and four weeks. Production of free radical may be due to gamma irradiation. When biological material is exposed to radiation, it quickly produces a large amount of reactive oxygen species (ROS), which are mostly produced by ionizing water molecules **(Reisz *etal., 2014).***

The oral administration of the milk thistle extract caused a significant reduction in free radicals in both blood and liver tissue of irradiated rats compared with positive control. The reducing of free radical may be due to the natural antioxidant compound (phenolic and flavonoid***)*** in milk thistle extract that acts as free radical scavenging. The achieved results agree with those reported by ***Natalia and Liudmyla (2021).***

Table 6. Effect of the milk thistle extract on Free Radicals in both blood and liver tissue of irradiated rats

Treatment / Groups	Free Radical in blood (Radicals/g)$^{\times 17}$		Free Radical in liver tissue(Radicals/g)$^{\times 17}$	
	Two days	Four weeks	Two days	Four weeks
-ve control	1.74±0.04	1.83±0.02^{b}	5.41±0.14	5.78±0.09^{b}
+ve control	2.28±0.03^{a}	3.45±0.05^{a}	60.84±0.79^{a}	40.57±0.64^{a}
MT	1.82±0.05^{ab}	1.87±0.06^{ab}	6.07±0.17^{b}	6.07±0.20^{b}
IR+MT	2.18±0.05^{a}	2.02±0.02^{b}	58.6±1.36^{a}	5.28±0.09^{b}

a Significant change with –ve control <0.05 ,b significant change with +ve control <0.05 at the same time, ,(MT) milk thistle(,IR) gamma irradiation

3.6 .2. The effect of milk thistle extracts on malondialdehyde (MDA) on irradiated rats

Table 7 demonstrates the effect of milk thistle extracts on malondialdehyde on irradiated rats after two days and four weeks, rats exposed to six gray gamma radiation. The results illustrated significantly higher levels of (MDA) has been occurred in their blood and liver tissue. The accomplished results are in agreement with that reported by ***Harry et al., (2023).*** According to these results, there is a direct link between ionizing radiation exposure and the degree of lipid peroxidation, which is indicated by MDA formation. Malondialdehyde (MDA), an aldehyde compound, is the result of lipid peroxidation in the body, which can occur through enzymatic or non-enzymatic processes ***(Jove et al., 2020).*** High concentrations of MDA suggest a process of oxidation occurring in the cell membrane, to defend against attacks by ROS ***(Aranda et al., 2020).***

An oxidative stress condition caused by an imbalance between antioxidants and free radicals in the body can arise because of elevated MDA concentration. The oral administration of the milk thistle extracts caused significant decrement (MDA) in serum and liver tissue of irradiated rats as shown in Table 7 which may be due to the natural antioxidant found in milk thistle plants. The achieved results are in agreement with that mentioned by ***(Nohair et al., 2017)***

Table 7. Effect of the milk thistle extract on malondialdehyde (MDA) in both serum and liver tissue of irradiated rats

Treatment / Groups	MDA in serum nmol/mL		MDA in liver tissue nmol/mg	
	Two days	Four weeks	Two days	Four weeks
-ve control	5.117±0.14	5.172±0.08^{b}	0.300±0.007	0.285±0.005^{b}
+ve control	11.914±0.15^{a}	9.635±0.15^{a}	0.455±0.006^{a}	0.402±0.006^{a}
MT	5.561±0.15^{b}	5.565±0.18^{b}	0.272±0.008^{ab}	0.272±0.010^{ab}
IR+MT	11.722±0.28^{a}	5.477±0.08^{b}	0.455±0.011^{a}	0.305±0.005^{b}

a Significant change with –ve control <0.05 ,b significant change with +ve control <0.05 at the same time, ,(MT) milk thistle(,IR) gamma irradiation

3.6 .3. The effect of milk thistle extracts on superoxide dismutase (SOD) in irradiated rats

After two days and four weeks, the rats exposed to six gray gamma irradiation showed significantly lower levels of SOD in serum and live tissue, as shown in Table 8 , and are in agreement with the results reported by ***Okeke et al., (2022)***. Who noticed that the observed decrement in the % inhibition of SOD might also be associated with the presence of excess ROS. SOD activity decline which is the most sensitive biomarker of liver damage and is a sign of hepatocellular injury. Enzyme molecules interact with these ROS, which denaturants and partially inactivate them. The antioxidant properties of phytochemicals like flavonoids and polyphenols found in milk thistle extract were responsible for the observed improvement in the enzymes SOD in the group that received milk thistle extract compared with the group that received radiation. Therefore, it has believed that the mechanism of action of milk thistle extract involves scavenging free radicals, donating hydrogen, and regulating endogenous enzymes. This outcomes are consistent with that noted by ***Nasser et al., (2023).***

Table 8. Effect of the milk thistle extract on superoxide dismutase activity *(* SOD*)* in both serum and liver tissue of irradiated rats

Groups	Treatment	Superoxide Dismutase (SOD) in serum U/Ml		Superoxide Dismutase (SOD in liver tissue U/mg tissue	
		Two days	Four weeks	Two days	Four weeks
-ve control		1561.00±22.3	1606.07±25.6[b]	1058.03±23.5	1055.17±16.8[b]
+ve control		1021.31±13.4[a]	1105.27±20.8[a]	231.55±2.9[a]	647.70±10.3[a]
MT		1578.38±46.4[b]	1579.11±53.0[b]	1042.89±29.5[b]	1043.53±35[b]
IR+MT		1021.75±22.1[a]	1540.45±32.1[b]	231.69±5.5[a]	994.59±29.1[b]

a Significant change with –ve control <0.05 ,b significant change with +ve control <0.05at the same time

3.6 .4. The effect of milk thistle extract on Glutathione (GSH) in irradiated rats

A six gray gamma irradiated showed significantly reduction in (GSH) in serum and liver tissue of rats after two days and four weeks as shown in Table 9 . The decrement in GSH concentration could be directly related to its use as an antioxidant that takes in free radicals produced by radiation. These results are in consistent with ***Okeke et al., (2022)***

The observed improvement in the level of GSH in serum and liver tissue in irradiated rats that received milk thistle extract. may be due to phenolic and flavonoid compound in milk thistle plant that act as natural antioxidant product in milk thistle extract which play as scavenging free radical. These results are in the same trend of the results reported by ***Natalia and Liudmyla(2021)***

Table 9. Effect of the milk thistle extract on Glutathione (GSH)) in both serum and liver tissue of irradiated rats

Groups	Treatment	Glutathione (GSH) in serum nmol/mL		Glutathione (GSH) in liver tissue nmol/mg	
		Two days	Four weeks	Two days	Four weeks
-ve control		4.635±0.12	4.970±0.08[b]	0.67±0.010	0.71±0.010[b]
+ve control		3.144±0.04[a]	2.840±0.04[a]	0.265±0.006[a]	0.04±0.00[a]
MT		5.26±0.14[ab]	5.260±0.17[ab]	0.759±0.023[ab]	0.76±0.026[ab]
IR+MT		3.146±0.07[a]	5.070±0.08[ab]	0.265±0.006[a]	0.73±0.010[ab]

a Significant change with –ve control <0.05 ,b significant change with +ve control <0.05at the same time , ,(MT) milk thistle(,IR) gamma irradiation

3.6 .5. The effect of milk thistle extracts on catalase (CAT) in irradiated rats

The gamma irradiation by six gray courses caused significantly decrement in the catalase enzyme activity in the serum and liver tissue of rats after two days and four weeks. as shown in Table 10. This dropping in enzyme activity may be due to the oxidative stress caused by production of free radicals after irradiation. The enzyme is consumed to directly interaction with free radicals. These results are in harmony with the data obtained by ***Okeke et al., (2022)***

Table 10 observes also improvement in enzyme catalase activity after receiving milk thistle extract orally for four weeks after irradiation. The improvement may be due to scavenging free radicals by phenolic and flavonoid compounds in milk thistle act as antioxidant products. The achieved results are in agreement with that noted by ***Natalia and Liudmyla (2021)***.

Table 10. Effect of the milk thistle extract on Catalase activates (CAT) in both serum and liver tissue of irradiated rats

Groups	Treatment	Catalase(CAT) in serum mg/dL		Catalase(CAT) in liver tissues mg/mg tissue	
		Two days	Four weeks	Two days	Four weeks
-ve control		8.42 ±0.11	8.09±0.11	14.71±0.19	14.9±0.23[b]
+ve control		5.16±0.06[a]	2.84±0.04[a]	9.29±0.12[a]	7.00±0.11[a]
MT		8.29±0.23[a]	8.32±0.27[b]	15.06±0.41[a]	15.07±0.50[b]
IR+MT		5.12±0.13[a]	7.91±0.13[b]	9.31±0.23[a]	13.79±0.21[b]

a Significant change with –ve control <0.05 ,b significant change with +ve control <0.05at the same time , ,(MT) milk thistle(,IR) gamma irradiation

Conclusion

Generally, from the abovementioned results it coulded be conclude that. The milk thistle ethanolic extract is rich in phenolic and flavonoid compounds. Thus it had a powerful antioxidant activity which might have resulted from the phenolic components' synergistic effects. In the present study milk thistle ethanolic extract showed antihyperlipidemic activities by powerful reactive oxygen scavenger through its antioxidant compounds, In addition also it enhanced liver and kidney functions compared with irradiated groups

Reference

Abdel-Mobdy A. E., El Hussieny M. S., and Abdel- Mobdy Y. E., (2021). Evaluation of therapeutic and protective influences of camel milk against gamma radiation– induced hematotoxicity, hepatotoxicity and nephrotoxicity in albino rats. Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, , Pages. 7958 – 7976

Aebi, H. (**1984**). Catalase in vitro Methods in Enzymology Volume 105Pages 121-126

Allain, C., C., Lucy, S. P., Cicely S. G., Chan, W. R., and Paul C. F. (1974). EnzymaticDeterminationof Total Serum Cholesterol CLIN. CHEM. 20/4, 470-475 *Photobiology B: Biology* Volume 178, January, Pages 489-495

Aranda.,R. A.K., Cruz,G. A., Arancibia,H. Y.L., Hernandez,C. E.Y. and Pedraza,C. J. (2020). ROS and oxidative stress: An overview of basic concepts. *Oxygen*, 2, 437-78.

Bartles, H., Böhmer, M. and Heierli, C. (1972).serum creatinine determination without protein precipitat ion Clin Chim Acta 37:193-7

Beutler E. and Mary K. Y. (1963). Erythrocyte glutathione reductase blood, VOL. 21, No. 5

Blois, M. S. (1958). Antioxidant determination by the use of a stable free radical. *Nature*, *181*(4617), 1199.

Costa, S.L., Silva, V.D.A., dos Santos Souza, C., Santos. C.C., Paris, I. and Munoz, P., (2016). Impact of plant- derived flavonoids on neurodegenerative diseases. Neurotox Res. 30(1):41-52

Daryoush, F. , Mohsen, M. , Babak ,S. , Arash, S. , Mohammad, S. and Ayoob, R.ade

(20 18). Radioprotective effects of Silymarin on the sperm parameters of NMRI mice irradiated with γ-rays. The *Journal of Photochemistry and Photobiology B: Biology* Volume 178, January, Pages 489-495

El-Dein, E., Anees, L.M. and Aly, S.M.E. (2016). Effects of a-lipoic acid on c-radiation and lindane-induced heart toxicity in rats. Pak J Zool. 48:1523–1529.

El-Hadary A. E., and Ramadan M.F. (2019). Phenolic profiles, antihyperglycemic, antihyperlipidemic, and antioxidant properties of pomegranate (Punica granatum) peel extract. J Food Biochem, ;43(4):e12803.

Elkady A. A., Rehab, M. E. and Wael, A. E. (2023) Eugenol Mitigates γ-Rays-induced Acute Renal Destructive Impacts in Rats *Egypt. J. Rad. Sci. Applic.* Vol. 36, No. 1-2, pp.

El-Shahat, A. N., Refaat, G. H., Ashraf, M. M. and Madeha, N. A.(2022). Ameliorative effect of graviola fruit juice on the damaged tissues of gamma-irradiated male rats Pakistan J. Zool., vol. 54(2), pp 583-590

El-Shennawy H.M., El- El Shahat, A.N., Ahmed, A.G.,and Nahed, A. (2016). Ameliorative effect of silymarin against radiation- Induced oxidative stress in the liver of male rats **,** pakestan j. zool., vol 48(6),pp.1905-1909

Fawcett, J.K. and Scott, J.E. (1960.) Arapid and precise method for the determination of urea. Journal of Clinical Pathology, 13, 156-159.

Federico, A.; Dallio, M.; Loguercio, C. (2017) silymarin/silybin and chronic liver disease: A marriage of many years. Molecules, 22, 191. [CrossRef] [PubMed]

Fossati, P. and Prencipe, L. (1982) serum triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chemistry, 28, 2077-2080.

Gohn, A; (1986).ESR and elementary particle applications, John, wiely. New Yourk,

Hamza, R.G., Mekawey, H.M.S., and El Shahat, A.N. (2013). Antioxidant activity of mulberry (Morus alba L.) fruits in male rats exposed to gammaradiation. Arab J Nucl Sci. 46:347–355.

Hanan, A. A., Salwa, N.A. and Mater, S. M. E.(2022) . Protective role of vitamin D against radiation hazards in rats fed on high fat diet Journal of Radiation Research and Applied Sciences 15 25–32

Harry, N. E., Surniyantoro,T. K., Devita, T., Darlina, Y., Iin Kurnia, H.B. and Yanti, L. (2023). Study of Immune response and malondialdehyde levels in irradiated rats supplemented with *curcuma xanthorriza* roxb extract *Asian Pacific Journal of Cancer Prevention, Vol 24*

Heckly. R. J., Harry ,N. E. S.,Teja,, K., Devita ,T. and Darlina Y. I. (1975). "Biological applications of electron spin resonance in free radicals in dry tissues. *Edited by: Swartz HM, Bolton. New York*: Wiley Inter ScienceJR, Borg DC: 5.

Ines, D. , Mourad, J. , Guido, F., Hazem, J., Mohamed, M. and Mohamed, B. (2020). Volatile and phenolic contents and antioxidant and antibacterial properties of tunisian milk thistle and mastic oils Euro-Mediterranean

Journal for Environmental Integration 5:59 https://doi.org/10.1007/s41207-020-00200-z

Ismaili, S.A., Marmouzi, I., Sayah, K., Harhar, H., Faouzi, M.E.A., Gharby, S., Himmi, B., Kitane, S., and Belghiti, M.A.E. (2016). chemical analysis and anti-oxidation activities of the moroccan milk thistle. Mor. J. Chem. 4(3): 695–702.

Janmejai, K. S. and Sanjay, G. (2009). Extraction, characterization sStability and biological activity of flavonoids isolated from chamomile flowers Mol Cell Pharmacol. January 1; 1(3): 138.

Javeed, A., Ahmed, M., Sajid, A.R., Sikandar, A., Aslam, M.,Hassan, T.u., Samiullah Nazir, Z.; Ji, M. and Li, C. (2022).comparative assessment of phytoconstituents, antioxidant activity and chemical analysis of different parts of milk thistle silybum marianum L. Molecules 27, 2641. https:// (doi.org/10.3390/ molecules27092641

Jove M., Mota-Martorell, N. and Pradas, I., (2020). The advanced lipoxidation end-product malondialdehyde-lysine in aging and longevity. *Antioxidants (Basel)*, **9**, 1-20.

Kim, H.G., Jang, S.S., Lee, J.S., Kim, H.S. and Son, C.G. (2017) Panax ginseng Meyer prevents radiation-induced liver injury via modulation of oxidative stress and apoptosis. J Ginseng Res 41:159–168. https:// doi.org/10.1016/j.jgr.2016.02.006

Klaus, R. , Maximilian ,N. and Bärbel, L.S. (2021) Radiation-induced kidney toxicity: molecular and cellular pathogenesis Radiation Oncology 16:43

Kurnia, H. B. and Yi, L. (2023). Study of immune response and malondialdehyde levels in irradiated rats supplemented with *curcuma xanthorriza* hoxb extract. *Asian Pac J Cancer Prev*, 24 (5), 1717-1723

Lucie, P. , Kristýna, K.A, David, B. , Vladimír, K.ˇ and Kateˇrina, V.(2020). simple and rapid HPLC separation and quantification of flavonoid,flavonolignans, and 2,3 dehydroflavonolignans in silymarin. foods, 9, 116

Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honey, as well as their radical scavenging activity. *Food Chemistry*, *91*, 571–577.

Mhamdi, B., Abbassi, F., Smaoui, A., Abdelly, C., and Marzouk, B. (2016). Fatty acids, essential oil and phenolics composition of Silybum marianum seeds and their antioxidant activities. Pak. J. Pharm. Sci. 29(3): 953–959

Moussa H.R., Mohammed, A.F.A., Osman, H.G. and Abd-Allah, M.A.N. (2015). Amelioration of oxidative stress induced by gamma irradiation in rats using spirulina platensis. Eur J Acad Essays. 2:90–97.

Naseer, M., Mohammed, H. F. A., Mukhallad, A. R., Uday, A. H., Ali, H. A. and Hassan T. A. *(* 2023*)*.Protective effect of silymarin on attenuating of rhabdomyolysis -induced acute kidney Injury in animal models Journal of Medical and Health Studies 0.32996/jmhs..4.6.5

Natalia, T. and Liudmyla, T. (2021). Antioxidant and anticytolytic action as the basis of the Pancreo-PlantR hepatoprotective effect in acute liver ischemia Čes. slov. Farm.; 70, 100–106

Nishikimi, M, Appaji, N. and Yagi. K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun., 46: 849-854

Nohair H., Sherif, A. M., Hawas, W. E. A., Ibrahim, A. S., Khaled A. S. and Faiza M. H. (2017).Otential role of milk thistle seed and its oil extracts against heart and brain injuries induced by γ-radiation exposure International Journal of Pharmacy and Pharmaceutical Sciences Vol 9, Issue 7, 52-58

Ohkawa, H. , Nobuko, O. and Kunio, Y.(1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction Analytical Biochemistry Volume 95, Issue 2, June, Pages 351-358

Okeke, S.I., Agwu ,K.K., Orji, M.P, Akpa, A.U., Okeke, C.H., and Anaga, A.O. (2022). Radioprotective effects of ethanolic extracts of *Gongronema latifolium* leaf against radiation induced oxidative stress in wistar albino rats International. Journal of Radiation Research, Volume 20, No 1

Ola S.M. A. , Nour E. A.,Salma M. A.and Omnia A.(2020) Ameliorative effect of kefir against γ-irradiation induced liver injury in male rats: impact on oxidative stress and inflammation . Environmental Science and Pollution Research 27:35161–35173

Porwal, O., Mohammed, A. M.S., Anwer, E.T., Uthirapathy, S., Ahamad, J. and Tahsin, A. (2019). silybum marianum (Milk Thistle): review on its chemistry, morphology, ethno medical uses, phytochemistry and pharmacological activities. J Drug Deliv Ther; 9(5):199-206.

Reisz, JA., Bansal, N., Qian, J., Zhao, W. and Furdui, C.M. (2014). Effects of ionizing radiation on biological molecules--mechanisms of damage and emerging methods of detection. Antioxid Redox Signal. PMID 10;21(2):260-92. doi: 10.1089/ars.2013.5489. Epub 2014 Feb 21.: 24382094; PMCID: PMC4060780.

Reitman ,S. and Frankel, S. (1957). Amer. J. clin. Path. 28, 56

Richmond, W. (1973) Preparation and Properties of a Cholesterol Oxidase from Nocardia sp. and Its

Application to the Enzy-matic Assay of Total Cholesterol in Serum. Clinical Chemistry, 19, 1350-1356

Saara, A., Asra, K. , Zehra, B. , Malik, H. M. , Saima, K. , Saiqa, T. , Shafiqa, N. R. and Saida H. (2020). Medicinal effects of saffron and chamomile on diabetes mellitus and associated hyperlipidemia and memory impairment Pak. J. Pharm. Sci., Vol.33, No.3(Suppl), May, pp.1191-1198

Saboon,S. K. Chaudhari,S. A.,Muhammad, S. A.,and Mohd S. A..(2019). Natural Bio-active compounds springer Nature Singapore Pte Ltd. 193, https://doi.org/10.1007/978-981-13-7154-7_7

SAS. (1996). *SAS Procedure Guide*. "Version 6.12 Ed". SAS Institute Inc., Cary, NC.

Satoh K.(1978). Serum lipid peroxides in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 90.; 37-43

Singleton, V. L., and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144–158.

***Spingo, G. and faveri, D.M.* (*2007*).** *Antioxidant from grabe stalks and mare influence of extraction procedure on ylied purity and antioxidant pow er of the extracts. J.of Food Eng , 78:793-801*

Tupe, R.S., Kemse, N.G., and Khaire, A.A. (2013). Evaluation of antioxidant potentials and total phenolic contents of selected Indian herbs powder extracts. Int. Food Res. J. 20(3): 1053–1063.

Vahid K., Maysam Mard S., Faraz M., Neda S.and Farhad N.(2023) The Effect of milk thistle, green tea, and cinnamon beverages on liver enzymes of operating room anesthesia personnel. Trends Med Sci. February; 3(1):e136000.

Weiss, J.F. and Lander, M.R., (2003). Protection against ionizing radiation by antioxidant nutrients and phytochemicals. *Toxicology*, **189**: 1-20. https://doi. org/10.1016/S0300-483X(03)00149-5

Xhuti D., Irena A. R., Mahek .M., Linda. M., Kieran, M., Joshua , P. N. and Mark A. (2023). TarnopolskyThe Acute Effect of Multi-Ingredient Antioxidant Supplementation following Ionizing Radiation Nutrients 15, 207. https:// doi.org/10.3390/nu15010207

Xiu,L., Dan,N., Jun,Q., Hong,Z. l. and Shao,K.(2009). Determination of silymarin flavonoids by HPLC and LC-MS and Investigation of extraction rate of silymarin in *silybum marianum* fruits by boiling water *Asian Journal of Chemistry* Vol. 21, No. 1 63-74

Zöllner, N. and Kirsch, K. (1962). Colorimetric method for determination of total lipids. Journal of Experimental Medicine, 135, 545-550.

تقيم تاثير مستخلص شوك الجمل علي حيوانات التجارب المعامله بأشعه جاما

زينب حامد حماد[2] ،ايناس محمود مكاوي [1]، محمد حسن عبد المجيد [2]،صلاح مصطفي محمود سعد[1]

1- قسم الكمياء الحيويه –كليه الزراعه –جامعه بنها

2- قسم المنتجات الطبيعيه –مركز تكنولوجيا الاشعاع–هيئه الطاقه الذريه – القاهره

تهدف هذه الدراسه الي دراسه تأثير المستخلص الكحولي لشوك الجمل علي تحسين وظائف الكبد والكلي وكذلك مستوي الدهون والكوليسترول وانزيمات الاكسده والجلوتاثيون والشقوق الحره ضد التلف الذي تسببه اشعه جاما . تم تعريض الجرذان الي اشعه جاما 6 جراي ثم اعطاءها المستخلص الكحولي شوك الجمل 200ملجم /كم من وزن الجسم . تم اخد عينات من دم الجرذان التجارب بعد يومان و اربعه اسابيع بعد التشعيع وتم تقدير وظائف الكبد والكلي وكذلك مستوي الدهون والكوليسترول وانزيمات الاكسده والجلوتاثيون والشقوق الحره.وقد وجد ان عند مقارنه النتائج في المجموعه التي تناولت المستخلص بدون التعرض للاشعاع ليس هناك فروق معنويه عند مقارنتها بالمجموعه الضابطه الغير مشعه , وقد ادي التشعيع الي زياده نشاط انزيمات الكبد ومستوي الكرياتينين واليوريا في السيرم وكذلك ارتفاع مستوي الدهون الكليه والجلسريدات الثلاثيه والكوليسترول الكلي زياده معنويه مقارنه بالمجموعه الضابطه الغير مشعه وكذلك نقص نشاط انزيما ت الاكسده يقابله زياده كبيره في مستوي الشقوق الحره والمالوندادي الدهيد , وكانت الزياده في النتائج بعد الاشعاع اربع اسابيع اكبر منها بعد يومين , كذلك اظهرت النتائج انخفاض معنوي في مستوي انزيمات الكبد ومستوي الكرياتينين واليوريا في السيرم وكذلك مستوي الدهون الكليه والجلسريدات الثلاثيه والكوليسترول الكلي في المجموعه التي تناولت مستخلص شوك الجمل بعد تشعيعها مقارنه بالمجموعه الضابطه المشعه.وزادت نشاط انزيمات الاكسده وانخفض تركيز الشقوق الحره والملوندادي الدهيد مقارنه بالمجموعه المشعه .وتوصي هذه الدراسه بانه من الممكن أستخدام مستخلص شوك الجمل للتقليل من الاضرار الناجمه عن الاشعاع .