Effect of Nano-extract of Silybum marianum seeds on liver enzymes and blood proteins in male albino rat induced liver cancer

Karrar Hussein Al-Kuraiti
Department of Biology - College of Education for Pure Sciences - University of Karbala, E-mail: karrarhussain886@gmail.com

Yasemin Khudair Alghanimi
Department of Biology - College of Education for Pure Sciences - University of Karbala

Rasha Abdul Amir Jawad
Department of Biology - College of Education for Pure Sciences - University of Karbala

Abstract

The current study aimed to find out the protective role of ZnO nanoparticles using Silybum marianum seeds on rats induced liver cancer by measuring some liver enzymes and blood proteins. In this study, Twenty five male white rats were used and divided into five groups. The first group, G1, was considered a negative control group, and the second group, G2, was considered a positive control group they were injected subperitoneal with thioacetamide (200mg/kg) for 14 days, then they were injected with CCl4 (3mg/kg). Once every week till the end of the experiment, while the remaining three groups were dosed with nano-extract at concentrations (15.8.5 mg/0.250kg) for 14 days, then injected subperitoneal with thioacetamide for 14 days, then injected with CCl4 once every week till end of the experiment. The study lasted for14 weeks. Blood samples and liver tissues were collected and the liver enzymes AST, ALT, ALP, albumin, globulin, and total protein were measured, the following results were discovered: A significant increase ($P<0.05$) in the level of the three liver enzymes (AST, ALT, ALP) for the positive control group compared to the negative control group. Also, the study showed that there were no significant differences ($P>0.05$) between the third group and the positive control group, but there was a decrease in significant level of liver enzymes in fourth and fifth groups comparing to the positive control group, and a significant decrease in the level of albumin, globulin and total protein of the positive control group comparing to the negative control group, but a significant increase occurred in groups G3, G4 and G5. Also the study showed that there were histological damage in G2 compared to the control group, but the damage was less in the other three group (G3,G4,G5) comparing to the positive control group .

Keywords: *Liver cancer, Thioacetamide, Silybum marianum, CCl4, Nanoparticle.*

INTRODUCTION

Cancer is defined as a state of abnormal division of cells due to a defect that affects the genetic material of those cells. It may affect any part of the human body and at any age, even fetuses in their mother's womb. The most important characteristics of the malignant disease are rapid spread, which often leads to death, with the possibility of a cure. if the disease's early detection and diagnosis are made, and despite medical development and progress, the causes that lead to the occurrence of the disease haven't known yet. Most cases are attributed to genetic mutations or a disorder in the DNA, there are

many factors that contributing to emergence of disease, including genetic predisposition, as well as environmental factors, including Postpartum exposure to chemicals, radioactive materials, and war remnants (Mustafa, 2020). Cancer disease is the second cause of death in humans worldwide, it caused nearly 9.6 million deaths in 2018 globally. It can be said that one person out of every 6 dies from cancer, which is nearly 70% of deaths from cancer. Cancer occurs in low- and middle-income countries (Bayrak et al., 2019). Chemotherapy has been the primary treatment option. However, chemotherapy has pharmacological limitations, including problems with stability and solubility in water, with non-specific toxicity to healthy cells, hair loss, anorexia, peripheral neuropathy and diarrhea as well as the emergence of multidrug resistance also a challenge. It is important for successful cancer treatment as cancer cells become resistant to many of the chemotherapy agents used (Awasthi et al., 2018). Numerous studies have focused on finding new therapies to reduce the side effects caused by conventional therapies. Nanomedicine offers a versatile platform of biocompatible and biodegradable systems capable of delivering conventional chemotherapy drugs in vivo, increasing their bioavailability and concentration around tumor tissues. Nanoparticles can be exploited for various applications, from diagnostics to therapeutics (Pucci et al., 2019). There are many types of cancer diseases, including liver cancer, which is one of the most common types of cancers of the gastrointestinal tract in the world, although there have been improvements in clinical treatments in recent years, but there have been no improvements in the diagnosis of affected patients, so there is an urgent need to identify biomarkers New predictive therapy for HCC so that treatment selection can be improved (Jiao et al., 2019). Liver cancer is usually caused by chronic liver disease, diabetes, alcoholism, non-alcoholic fatty liver disease as well as viral hepatitis. There are many methods used to treat liver cancer, including surgical removal and non-surgical treatments. selective on disease stage, liver function, and treatment cost. Although the survival of patients with HCC is prolonged, recurrence remains a major problem in the treatment of HCC (Zhang et al., 2020). Medicinal plants have been used since ancient times and can be considered the origin of modern medicine. Plant-based compounds were and still are important sources of medicinal compounds. In the beginning, the method of trial and error was used to treat diseases or even just feel better to distinguish between plants with beneficial effects. The use of these plants has been gradually improved. Over the generations the use of plant has become known in many contexts as traditional medicine and the healing properties of plants have led to the emergence of medicinal drugs that are made of specific plants with these benefits (Salmerón-Manzano et al., 2020). The Iraqi lands are rich in medicinal plants, since ancient times the Iraqi people have trusted healing in medicinal plants. One of the most important of these medicinal plants growing naturally in all parts of Iraq is the kharfish plant, or what is known as (Kalgan), as it was used a lot as a tonic for the liver in ancient Greek and Arab medicine, which was later contributed to studies. Scientific and clinical studies, which made this plant at the top of plants used in regeneration of liver cells and treatment of diseases (Eldalawy & Al-Ani, 2020). The herb Silybum marianum belongs to the Compositae family. It is an annual plant native to the Mediterranean region. It is widespread in warm and dry regions. It also grows in Iraq, especially the north. The most important medical application of the herb is its use as a hepatoprotector and as a supportive treatment for chronic inflammatory liver disorders such as cirrhosis, hepatitis and substances. Toxic chemical such as lead, as silymarin derived from the milk thistle plant

was widely used in the self-medication of liver diseases as well as used to treat liver cancer (Shahab, 2019).

MATERIALS AND METHODS

Animals

Twenty five adult male white rats weighting 200-250 g (10-12 weeks)are used. they were brought from the Animal House of the College of Pharmacy - Karbala University. They were placed in plastic cages with metal caps for rat breeding The floor of the cages was sprinkled with sawdust, with occasional changes. Water and food of the animal feed were provided under suitable ventilation conditions at a temperature of 25 ° C, with 12 hours light /dark cycle The animals were allowed to adapt for two weeks before starting the experiment.

Experimental design

In the current study, (25) male white rats were randomly divided into five groups, with 5 animals per group, as follows:

1- The first group: the control group is given only normal saline

2- The second group: the infection group (positive control) was injected with Thioacetamide (TAA) at an amount of (200 mg/kg) dissolved at a rate of 0.9 and given for two weeks, after which it was injected with CCL4 at an amount of (3mg/kglweek) for a period of 6 weeks to induce cancer

3- The third group: they were dosed with the nanoextract) 5 mg/0.250 kg) for two weeks, after which the mixture of TAA + CCl4 was given till the end of the experiment, which was 14 weeks.

4- The fourth group: they were dosed with the nanoextract (8 mg/0.250 kg) for two weeks, after which the mixture of TAA + CCl4 was given till the end of the experiment, which was 14 weeks.

5- The fifth group: they were dosed with the nanoextract (15 mg/0.250 kg) for two weeks, after which the mixture of TAA + CCl4 was given till the end of the experiment, which was 14 weeks.

Inducing of Liver Cancer

Liver cancer was induced in male rats in two stages: the first stage was injected subperitoneally with thioacetamid at a dose of (300 mg/kg) after it was dissolved in distilled water and given for two weeks, it has been proven that the substance is toxic and carcinogenic to the liver (Anwer & Baker, 2021). TAA is an organic compound used in leather processing, in laboratories, and in the textile and paper industries. It is a typical hepatotoxic substance consumed to induce acute and chronic liver injury due to its effects on protein synthesis, RNA, and DNA. Prolonged administration leads to large liver nodules, hepatocellular adenomas, and cholangiocarcinomas. and liver tumors (Akhtar & Sheikh, 2013).

Preparation of the nano-extract

Prepare the nano-extract by adding 1 gm of zinc oxide to 50 ml of deionized distilled water, then add 1 gm of alcoholic extract of the Artichoke plant, then stir the mixture with a magnetic stirrer at room temperature for a full day (24 hours), then put The mixture in the shaker incubator at a temperature of 40°C for 18 hours then the precipitate is separated by a centrifuge at a speed of 3000 cycles for 20 minutes, after is washed with distilled water deionized several times, then the precipitate is dried in an electric oven at a temperature of 50°C, then it is ground with a ceramic mortar well to obtain a fine powder. This powder is kept in the refrigerator till it is used. The method described was that of Bashi et al., (2013).

Collection of The Blood 's Samples

After starving the animals for 12 hours, blood samples were collected by anesthetizing the animals using chloroform anesthesia by placing a cotton ball containing the anesthetic substance in a large box in which the animal was present to be anesthetized by breathing, then 5 ml of blood was withdrawn directly from the heart by stabbing the heart. puncture to obtain the largest amount of blood, put 2 ml of blood samples in test tubes (gel tube) then placed in the centrifuge at a speed of 3000 rpm for 10 minutes, after the separated serum was transferred to small plastic tubes marked clean and dry, after that, these tubes containing the serum were kept in a state of freezing at a low temperature estimated at -20 °C till the liver enzymes tests were performed on them later.

Histological samples collection

After the animals were anesthetized by chloroform and blood samples were drawn from them, the animals were dissected and the liver was removed from the animal's body, then it was washed with water to remove the blood on it, then it was dried by placing it on filter paper, these organs were cut into small pieces longitudinally and transversely in order to be preserved with ensuring that the preservative has reached it, and the samples are kept in clean and dry labeled containers with tight lids containing 10% Formalin, and then these containers are left until tissue cutting is performed on them. Where the histological cutting process was done depending on the method (Suvarna et al., 2013)

Measurement of liver enzyme activity

The activity level of ALT and AST enzymes in rat serum was measured using a ready-made assessment kit and according to the method used by (Bergmeyer & Rej, 1985),while for the estimation of the activity level of the ALP enzyme using an enzymatic method based on the method of (Belfield & Golderg, 1971)which is a colorimetric method based on the use of the substrate on which the alkaline phosphatase enzyme works.

Measurement of serum proteins

The ready-made test kit was used to measure the level of total protein in the blood serum, which is an enzymatic method based on the use of the Biuret reagent (Naithani & Singh, 2006), while the albumin level was measured in the blood serum, depending on the method of (Friedman & Young, 2001). The concentration of globulin protein in blood serum was extracted by subtracting the previously extracted albumin protein value from the total protein value of all samples, according to the following equation (Scimone & Rothstein, 1978). Globulin concentration (g/dl) = total protein concentration - albumin concentration

STATISTICAL ANALYSIS

Statistical analysis was carried out for an experiment of 5x5 replications according to a complete randomized design to study the effect of nano-extract of Silybum marianum seeds on the studied parameters and to test the significance of the differences between the means using (L.S.D) Revised Least Significant Differences by means of SPSS program.

RESULTS

Liver Enzymes

The results of table (1) showed that there was a significant increase ($P<0.05$) in the level of AST enzyme (113.80 ± 3.541) in the G2 positive control group in which hepatocellular carcinoma induced comparison to the G1 negative control group (68.80 ± 2.764) that was not given any substance, while there was a decrease Significant ($P<0.05$) in the other three groups G3, G4 and G5 that were dosed with ZnO nanoparticles using Silybum marianum seeds (5mg, 8mg, 15mg) (101.80 ± 3.639) (80.60 ± 3.187) (81.40 ± 3.092)

respectively comparison to the positive control group G2, but this decrease it did not reach what it is in the negative control group G1, while the results showed that there were no significant differences (P<0.05) between the two groups G5, G4. The results of table (1) showed that there was a significant increase (P < 0.05) in the level of ALT enzyme (60.80 ± 2.107) in the positive control group, G2, in which liver cancer induced, comparison to the negative control group, G1 (30.60 ± 2.159), which was not given any substance, while there were differences Significant in the other three groups G3, G4 and G5 that were dosed with ZnO nanoparticles using Silybum marianum seeds (5mg, 8mg, 15mg) (53.80 ± 1.655) (43.00 ± 1.817) (44.20 ± 1.594) respectively comparison to the positive control group G2, but this decrease did not reach what is in the negative control group G1, whereas the results showed that there are no significant differences (P < 0.05) between the two groups G5, G4. The results of table (1) showed that there was a significant increase (P < 0.05) in the level of ALP enzyme (266.40 ± 2.441) in the G2 positive control group in which hepatocellular carcinoma induced comparison to the G1 negative control group (180.40 ± 4.069) that was not given any substance, while there was a decrease Significant (P<0.05) in the other three, G3, G4, and G5 that were dosed with ZnO nanoparticles using Silybum marianum seeds (15mg, 8mg, 5mg) (255.40 ± 4.226) (215.60 ± 2.993) (219.80 ± 2.518) respectively comparison to the positive control group G2, but this decrease did not reach to what it is in the negative control group G1, whereas the results showed that there are no significant differences (P<0.05) between the two groups G5, G4.

Table (1) Effect of treatment with ZnO nanoparticles using Silybum marianum seeds on the level of liver enzymes AST, ALT and ALP (U/L) in the blood serum of white male rats induced with liver cancer.

Treatments	**AST** (U/L)	**ALT** (U/L)	**ALP** (U/L)
Negative control group	**68.80 ± 2.764 A**	**30.60 ±2.159 A**	**180.40 ±4.069 A**
positive control group	**113.80 ± 3.541 B**	**60.80± 2.107 B**	**266.40± 2.441 B**
TAA+CCl4/NP extract(5mg/kg)	**104.40 ± 3.124 C**	**53.80 ±1.655 C**	**255.40 ±4.226 C**
TAA+CCl4/NP extract(8mg/kg)	**80.60 ± 3.187 D**	**43.00 ±1.817 D**	**215.60 ±2.993 D**
TAA+CCl4/NP extract(15mg/kg)	**81.40 ± 3.092 D**	**44.20 ±1.594 D**	**219.80 ±2.518 D**

Average ± standard error. n=5. The different capital letters in the vertical direction indicated to significant differences P>0.05

Blood Proteins

The results of table (2) showed a significant decrease (P<0.05) in the level of albumin (2.270 ± 0.044) in the positive control group, G2, in which hepatocellular carcinoma induced, comparison to the negative control group, G1 (4.222 ± 0.136), which was not given any substance, while a significant increase was found (P<0.05) in the other three groups G5, G6, and G7 that were dosed with ZnO nanoparticles using Silybum marianum seeds (5mg, 8mg, 15mg) (2.920 ± 0.124) (3.432 ± 0.180) (3.566 ± 0.078) respectively comparison to the positive control group G2, but this decrease did not reach to what it is in the negative control group G1, where the results showed that there are no significant

differences (P<0.05) between the two groups G5, G4. Also, the results of table (2) showed a significant decrease (P<0.05) in globulin level (1.468 ± 0.069) in the G2 positive control group in which hepatocellular carcinoma induced comparison to the G1 negative control group (2.480 ± 0.138) that was not given any substance, while a significant increase was found. (P<0.05) in the other three groups G3, G4, and G5 that were dosed with ZnO nanoparticles using Silybum marianum seeds (5mg, 8mg, 15mg) (2.018 ± 0.096) (2.272 ± 0.061) (2.378 ± 0.057) respectively comparison to the positive control group G2, but this decrease was not it reaches what it is in the negative control group G1 except for group G5, whereas the results showed that there are no significant differences (P<0.05) between the two groups G5, G4. The results of table (2) showed that there was a significant decrease (P<0.05) in the level of total protein (3.738 ± 0.084) in the positive control group, G2, in which hepatocellular carcinoma induced, comparison to the negative control group, G1 (6.702 ± 0.216), which was not given any substance, while a significant increase was found. (P<0.05) in the other three groups G3, G4, and G5 that were dosed with ZnO nanoparticles using Silybum marianum seeds (5mg, 8mg, 15mg) (4.938 ± 0.154) (5.704 ± 0.157) (5.944 ± 0.062) respectively comparison to the positive control group G3, but this decrease was not it reaches what it is in the negative control group G1, while the results showed that there are no significant differences (P < 0.05) between the two groups G5, G4.

Table (2) Effect of treatment with ZnO nanoparticles using Silybum marianum seeds on the level of Blood Proteins (Albumin, Globulin and Total Protein) (g/dl) in the blood serum of white male rats induced with liver cancer.

Treatments	Albumin (**g/dl**)	Globulin (**g/dl**)	Total Protein (**g/dl**)
Negative control group (G1)	**4.222 ± 0.136 A**	**2.480 ± 0.138 A**	**6.702 ± 0.216 A**
positive control group (G2)	**2.270 ± 0.044 B**	**1.468 ± 0.069 B**	**3.738 ± 0.084 B**
TAA+CCl4/NP extract(5mg/kg) (G3)	**2.920 ± 0.124** C	**2.018 ± 0.096** C	**4.938± 0.154** C
TAA+CCl4/NP extract(8mg/kg) (G4)	**3.432 ± 0.180** D	**2.272 ± 0.061** D	**5.704 ± 0.157** D
TAA+CCl4/NP extract(15mg/kg) (G5)	**3.566 ± 0.078** D	**2.378 ± 0.057** AD	**5.944 ± 0.062** D

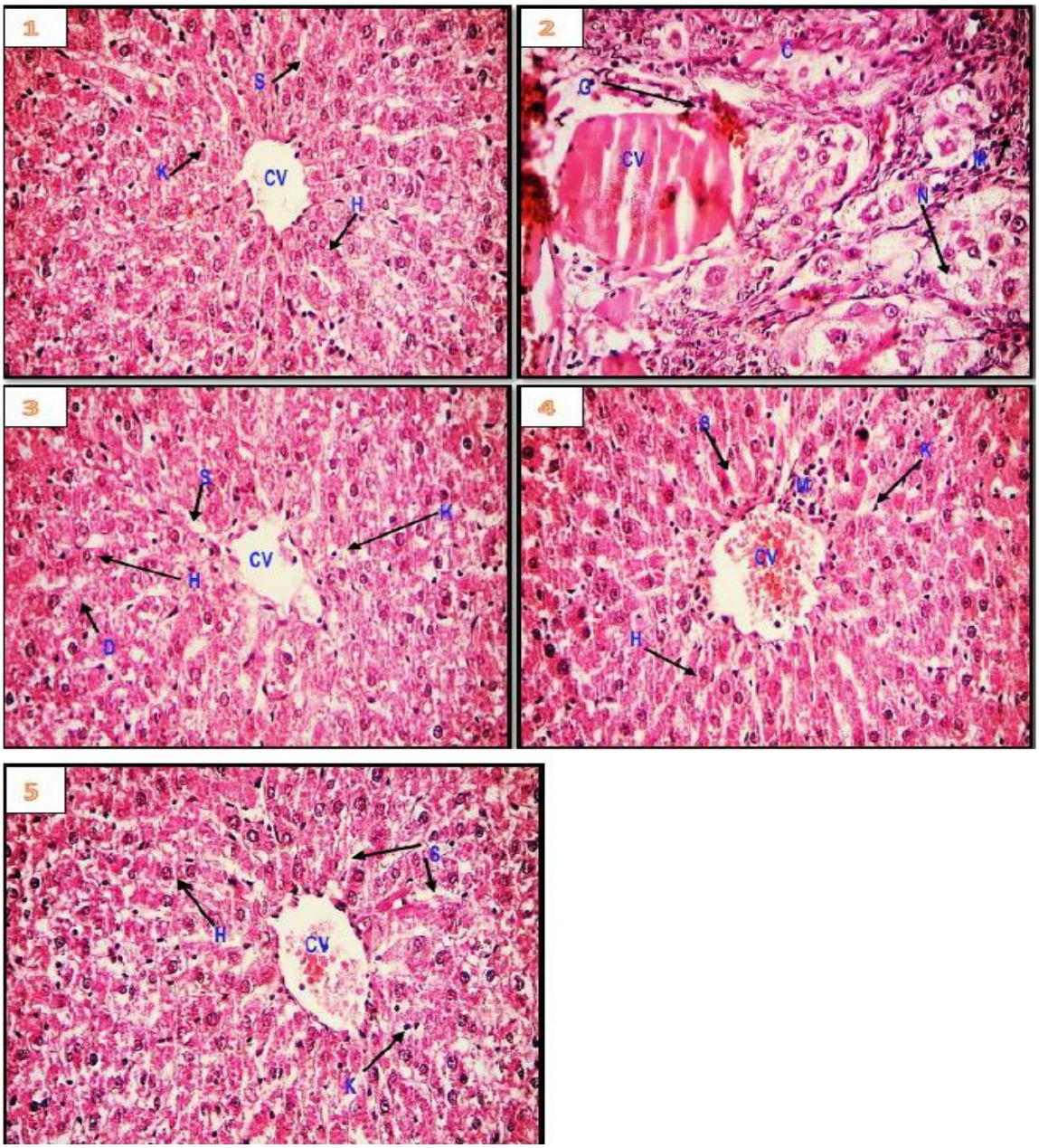
Average ± standard error. n=5. The different capital letters in the vertical direction indicated to significant differences P>0.05

Liver tissues

Image (1) referred to a cross section of the liver tissue of the normal control group showing normal hepatocytes and the central vein in addition to sinusoids and Kupffer cells, and image (2) was a cross section of the liver tissue of the positive control group in which tumor cells and necrosis were observed in the liver cells with hemorrhage In the tissue, the sinusoids are irregular, as well as irregular in the shape of the hepatic lobule. The section shows inflammation in some areas of the tissue when compared with image (1) of the negative control group. Image (3) shows a cross-sectional section of the liver tissue of the third group (nanoextract 20mg/kg + liver cancer induction Where there is

decomposition in some liver cells with regularity of sinusoids, as well as normal hepatocytes, central vein and Kupffer cells appear compared with image (2) of the positive control group, while image (4) indicated a cross-sectional section of the liver tissue of the fourth group (nano extract 32mg/kg + induction of liver cancer) in which there is mild inflammation in the tissue and the central liver vein, sinusoids, hepatocytes, and Kupffer cells are also shown. Image (5) was for the fifth group (nanoextract 60mg/kg + induction of hepatocellular carcinoma). liver) and the central vein, hepatocytes, sinusoids, and Kupffer cells appear normally.

Fig (1)- control group (2)- positive control group (3)- third group (4)- fourth group (5)- fifth group, (CV) central vein (S) sinusoids (M) inflammation (H) Hepatocyte (K) Kupffer cells (G) hemorrhage (D) Degeneration



DISCUSSION

The results of the current study revealed that the induction of liver cancer with thioacetamide (TAA) and carbon tetrachloride (CCl4) caused an increase in the level of liver enzymes, and this agreed with the results of the study (Anwer & Baker, 2021) and (khalaf, 2021). It is known that TAA disrupts the processes of hepatocellular metabolism and stimulates oxidative stress that leads to liver necrosis, this can be clarified that TAA-induced lipid peroxidation leads to damage to hepatocytes or necrosis, and this contributes to the secretion of enzymes and then elevation of the level of liver enzymes in the blood serum, which is an indicator of hepatocellular necrosis caused, about TAA metabolism that caused hepatocyte damage (Hussein et al., 2020). As for liver damage caused by CCl4, CCl4 metabolism may begin with the formation of free radicals through the activity of the oxygenase system in cytochrome P450 in the endoplasmic reticulum, whereas these free radicals interact with many important biological materials such as fatty acids, proteins, lipids, nucleic acids, and amino acids, disturbing the balance between the production of reactive oxygen species and the system of reactive oxygen species antioxidant defense due to oxidative stress that disrupts cellular functions by some event and causes liver damage and necrosis (Mahmoodzadeh et al., 2017). The study showed a decrease in the level of liver enzymes in the groups that were dosed with the ZnO nanoparticles using Silybum marianum seeds and this is inline with the results of the study of (El Hassanen et al., 2021) and (Feng et al., 2019). Silybum marianum is a well-known plant that is used as medicine and food and has been widely used to treat various diseases, especially liver diseases, the seeds and fruits of Silybum marianum contain a flavonolignan compound called silymarin, and active compounds that include silybin, isosilybin, silychristin, dihydrosillin, silydianin and these active compounds in the Silybum marianum has a significant role in relieving the oxidative stress that hepatocytes are exposed to (Wang et al., 2020). Studies have shown that silymarin has potential effects on the liver due to its antioxidant and anti-inflammatory effects, cellular membrane stabilization, promotion of hepatocyte regeneration, and inhibition of fibrosis furthermore, silymarin is considered a supportive therapy to protect the liver from toxicity caused by chemicals, and it has significant effects in eliminating reactive oxygen species and can reduce drug toxicity (Tajmohammadi et al., 2018). Silymarin appears to inhibit the uptake of toxins by binding to transmembrane receptors in hepatocytes which alters the structure of the transmembrane receptor, Conversely, the antioxidant properties of silybum marianum prevent metabolic disturbances of these cells by inhibiting lipid peroxidation in hepatocytes and silymarin also has a positive effect on preventing altered lipoprotein metabolism which is a major cause of cellular and metabolic problems (Zaker ‐ Esteghamati et al., 2021). Also, vitamin E is the active ingredient in silybum marianum with antioxidant, anti-inflammatory, and anti-apoptotic properties and the use of vitamin E has been found to reduce liver enzymes With improved biochemical properties and histological examination revealed that vitamin E intake improves lobular inflammation and hepatic steatosis (Jiang et al., 2022).

The induction of liver cancer with thiostamide (TAA) and carbon tetrachloride (CCl4) decreased the level of blood proteins (albumin, globulin, and total protein), and this is consistent with the results of (Abd-Elrahman et al., 2014) and (Rahman et al., 2020). It is known that the liver is mainly responsible for the formation of blood proteins, and that a decrease in their percentage in blood serum is evidence of liver injury (Jayesh et al., 2019). Also, thioacetamide has been used for years in

causing liver toxicity, this toxicity is due to the activity of oxidative stress caused by TAA and damage to liver cells, and single doses of it cause central necrosis (El-Deberky et al., 2021). While treatment with the ZnO nanoparticles using Silybum marianum seeds caused an increase in the level of blood proteins, and this is consistent with the study (El-Ghany et al., 2022). Some studies have reported that silymarin and silibinin achieve reduction of visceral fat retention by inhibiting lipogenic gene expression and/or improve lipolysis and plasma lipoprotein content through regulation of plasma lipoproteins. Silymarin acts as an antioxidant due to its anti-inflammatory effects and has been used in the treatment of several liver damages (Hassaan et al., 2019).

Histological sections showed that the induction of liver cancer with thioastamide (TAA) and carbon tetrachloride (CCl4) caused histological changes in the liver tissue, and this is agree with the results of the study (Algandaby, 2018). Hepatotoxic and carcinogenic effect by causing cell enlargement, TAA is metabolized to thioacetamide-S-oxide and acetamide once administered to rats, thioacetamide-S-oxide binds to macromolecules in the cell responsible for the change in cell permeability and calcium uptake, this interruption in calcium stores leads to enlargement of nuclei, and inhibition of mitochondrial activity eventually leading to hepatic necrosis (Zargar et al., 2017). CCl4 promotes the secretion of several inflammatory cytokines and their metabolites form trichloromethyl and trichloromethyl peroxyl radicals, these byproducts are unstable, which in turn bind to protein or lipids and cause lipid peroxidation, which leads to damage to the cell membrane, this contributes to liver injury and causing hepatocyte apoptosis (Yan et al., 2019). Treatment with the ZnO nanoparticles using Silybum marianum seeds caused an improvement in liver tissue, and this is consistent with the results of the study (Khalili et al., 2021). Milk thistle plant exhibits its hepatoprotective properties through three main mechanisms, firstly, it acts as an antioxidant, secondly as an anti-inflammatory, and thirdly, as an antifibrotic substance, the anti-inflammatory properties are attributed to its ability to regulate cytokines responsible for inducing inflammation, the plant has been shown to regulate and inhibit the expression of COX-2, a key mediator of inflammatory pathways (Achufusi & Patel, 2021).

Reference

Abd-Elrahman, N. M., Farid, A. S., & Fararh, K. M. (2014). Effect of gentamicin and thioacetamide toxicity on serum proteins. BENHA VETERINARY MEDICAL JOURNAL, 27 (2), 456 - 465.

Achufusi, T. G. O., & Patel, R. K. (2021). Milk Thistle. In StatPearls [Internet]. StatPearls Publishing.

Akhtar, T., & Sheikh, N. (2013). An overview of thioacetamide-induced hepatotoxicity. Toxin Reviews, 32(3), 43–46.

Algandaby, M. M. (2018). Antifibrotic effects of crocin on thioacetamide-induced liver fibrosis in mice. Saudi Journal of Biological Sciences, 25(4), 747–754.

Anwer, M. I., & Baker, L. A. A. (2021). Effect of Annona Muricata Extracts on Some Biochemical Parameters in Rats Exposed to Induced Liver Tumor. JOURNAL OF EDUCATION AND SCIENCE, 30(1), 72–88.

Awasthi, R., Roseblade, A., Hansbro, P. M., Rathbone, M. J., Dua, K., & Bebawy, M. (2018). Nanoparticles in cancer treatment: opportunities and obstacles. Current Drug Targets, 19(14), 1696–1709.

Bashi, A. M., Hussein, M. Z., Zainal, Z., & Tichit, D. (2013). Synthesis and controlled release properties of 2, 4-

dichlorophenoxy acetate–zinc layered hydroxide nanohybrid. Journal of Solid State Chemistry, 203, 19–24.

Bayrak, E. A., Kırcı, P., & Ensari, T. (2019). Comparison of machine learning methods for breast cancer diagnosis. 2019 Scientific Meeting on Electrical-Electronics & Biomedical Engineering and Computer Science (EBBT), 1–3.

Belfield, A., & Golderg, G. M. (1971). Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine-Enzyme. 12, 561–573.

Bergmeyer, H. U., & Rej, M. H. and R. (1985). INTERNATIONAL FEDERATION OF CLINICAL CHEMISTRY (IFCC) Scientific Committee, Analytical Section. J. Clin. Chem. Clin. Biochem., 24, 481–495.

El-Deberky, D., Rizk, M., Elsayd, F., Amin, A., & El-Mahmoudy, A. (2021). Protective potential of Cynara scolymus extract in thioacetamide model of hepatic injury in rats. 6(2). http://dx.doi.org/10.21931/RB/2021.06.02.20

El-Ghany, A., Nagib, R. M., & Hashem, H. A. (2022). Hepatotreatment Effect of Silybum, Spirulina and Propolis Consumption Compared to Silymarin Drug in Experimental Rats. Journal of Food and Dairy Sciences, 13(1), 17–24.

El Hassanen, Y. A., Badran, H., Abd EL-Rahman, A. N. A., & Badawy, N. M. (2021). Potential Effect Of Milk Thistle (Silybum Marianum) On Liver Disorders Induced By Carbon Tetrachloride. Journal of Home Economics, 31(1), 83–93.

Eldalawy, R., & Al-Ani, W. M. K. (2020). GC-MS analysis of Iraqi Silybum marianum Flowers, Leaves and Seeds Extracts. Al Mustansiriyah Journal of Pharmaceutical Sciences, 20(4), 93–112.

Feng, R., Chen, J.-H., Liu, C.-H., Xia, F.-B., Xiao, Z., Zhang, X., & Wan, J.-B. (2019). A combination of Pueraria lobata and Silybum marianum protects against alcoholic liver disease in mice. Phytomedicine, 58, 152824.

Friedman, & Young. (2001). Effects of disease on clinical laboratory tests (4th ed.). AACC press.

Hassaan, M. S., Mohammady, E. Y., Soaudy, M. R., El-Garhy, H. A. S., Moustafa, M. M. A., Mohamed, S. A., & El-Haroun, E. R. (2019). Effect of Silybum marianum seeds as a feed additive on growth performance, serum biochemical indices, antioxidant status, and gene expression of Nile tilapia, Oreochromis niloticus (L.) fingerlings. Aquaculture, 509, 178–187.

Hussein, R. M., Anwar, M. M., Farghaly, H. S., & Kandeil, M. A. (2020). Gallic acid and ferulic acid protect the liver from thioacetamide-induced fibrosis in rats via differential expression of miR-21, miR-30 and miR-200 and impact on TGF-β1/Smad3 signaling. Chemico-Biological Interactions, 324, 109098.

Jayesh, K., Helen, L. R., Vysakh, A., Binil, E., & Latha, M. S. (2019). Protective role of Terminalia bellirica (Gaertn.) Roxb fruits against CCl4 induced oxidative stress and liver injury in rodent model. Indian Journal of Clinical Biochemistry, 34(2), 155–163.

Jiang, G., Sun, C., Wang, X., Mei, J., Li, C., Zhan, H., Liao, Y., Zhu, Y., & Mao, J. (2022). Hepatoprotective mechanism of Silybum marianum on nonalcoholic fatty liver disease based on network pharmacology and experimental verification. Bioengineered, 13(3), 5216–5235.

Jiao, Y., Li, Y., Fu, Z., Hou, L., Chen, Q., Cai, Y., Jiang, P., He, M., & Yang, Z. (2019). OGDHL expression as a prognostic biomarker for liver cancer patients. Disease Markers, 2019.

khalaf, H. M. (2021). Hepatoprotactive activity of Ruta chalepensis ethanolic extract and histo-architecture of liver on CCL 4 damaged albino male mice. Journal of Biotechnology Research Center, 15(1), 11–16.

Khalili, A., Fallah, P., Hashemi, S. A., Ahmadian-Attari, M. M., Jamshidi, V., Mazloom, R., Beikzadeh, L., & Bayat, G. (2021). New mechanistic insights into hepatoprotective activity of milk thistle and chicory quantified extract: The role of hepatic Farnesoid-X activated receptors. Avicenna Journal of Phytomedicine, 11(4), 367–379. https://doi.org/10.22038/AJP.2020.17281

Mahmoodzadeh, Y., Mazani, M., & Rezagholizadeh, L. (2017). Hepatoprotective effect of methanolic Tanacetum parthenium extract on CCl4-induced liver damage in rats. Toxicology Reports, 4, 455–462.

Mustafa, S. W. (2020). Environmental pollution and its impact on the increase in child cancer cases in Basra Governorate. Journal of Basra Researches for Human Sciences, 45(4).

Naithani, M., & Singh, P. (2006). Teitz Textbook of Clinical Chemistry & Molecular Diagnostics. In Medical Journal, Armed Forces India (Vol. 62, Issue 2, p. 204).

Pucci, C., Martinelli, C., & Ciofani, G. (2019). Innovative approaches for cancer treatment: Current perspectives and new challenges. Ecancermedicalscience, 13.

Rahman, F., Sarma, J., Mohan, P., Barua, C. C., Nath, R., Rahman, S., & Sarma, M. (2020). Role of Nano-curcumin on carbon tetrachloride (CCl4) induced hepatotoxicity in rats. Journal of Pharmacognosy and Phytochemistry, 9(2), 2168–2176.

Salmerón-Manzano, E., Garrido-Cardenas, J. A., & Manzano-Agugliaro, F. (2020). Worldwide research trends on medicinal plants. International Journal of Environmental Research and Public Health, 17(10), 3376.

Scimone, J., & Rothstein, R. (1978). Laboratory Manual of Clinical Chemistry. AVI publishing company,Inc., U.S.A.

Suvarna, S. K., Layton, C., & Bancroft, J. D. (2013). Bancroft's Theory and Practice of Histological Techniques. In Elsevier (pp. 11–36). https://doi.org/10.1016/B978-0-7020-4226-3.00002-0

Tajmohammadi, A., Razavi, B. M., & Hosseinzadeh, H. (2018). Silybum marianum (milk thistle) and its main constituent, silymarin, as a potential therapeutic plant in metabolic syndrome: A review. Phytotherapy Research, 32(10), 1933–1949.

Wang, X., Zhang, Z., & Wu, S.-C. (2020). Health benefits of Silybum marianum: Phytochemistry, pharmacology, and applications. Journal of Agricultural and Food Chemistry, 68(42), 11644–11664.

whap Shahab, Z. A. (2019). ALcoholic extraction of silymarin oil from seeds of local Milk thistle plant (silynum mariamum) at room temperature. مجلة الكوفة للعلوم الطبية البيطرية| Kufa Journal For Veterinary Medical Sciences, 10(2).

Yan, Y., Jun, C., Lu, Y., & Jiangmei, S. (2019). Combination of metformin and luteolin synergistically protects carbon

tetrachloride‐induced hepatotoxicity: Mechanism involves antioxidant, anti‐inflammatory, antiapoptotic, and Nrf2/HO‐1 signaling pathway. Biofactors, 45(4), 598–606.

Zaker‐Esteghamati, H., Seidavi, A., & Bouyeh, M. (2021). The effects of Cynara scolymus and Silybum marianum on growth, carcass and organ characteristics, immunity, blood constitutes, liver enzymes, jejunum morphology, and fatty acid profile of breast meat in broilers. Food Science & Nutrition, 9(12), 6692–6706.

Zargar, S., Wani, T. A., Alamro, A. A., & Ganaie, M. A. (2017). Amelioration of thioacetamide-induced liver toxicity in Wistar rats by rutin. International Journal of Immunopathology and Pharmacology, 30(3), 207–214. https://doi.org/10.1177/0394632017714175

Zhang, C., Yang, M., & Ericsson, A. C. (2020). The potential gut microbiota-mediated treatment options for liver cancer. Frontiers in Oncology, 10, 524205.