Original Article

Evaluation of The Efficiency of Milk Thistle (Silybum marianum) Against Hazardous Effects of Ethylene Glycol in Male Albino Rats

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ABSTRACT

Introduction: Ethylene glycol, a common ripening agent for fruits can induce oxidative damage to healthy tissues, which can lead to organ failure. However, milk thistle, a plant with traditional medicinal use, can eliminate harmful free radicals and regulate inflammatory reactions.

Aim of the Work: In this work, the efficacies of milk thistle aqueous extract to mitigate the detrimental effects of ethylene glycol on the organs of male albino rats were explored.

Materials and Methods: Four groups of forty rats were allocated as control group (G1), milk thistle extract treated group (G2) received a daily intake of 151.2 mg/kg milk thistle for 1month, G3 received ethylene glycol to induce toxicity (2 ml/kg/day for 3 days), and G4 received both ethylene glycol and milk thistle extract. Biochemical analysis for blood samples as liver function (ALT, AST and ALP), total protein and albumin, kidney function (urea and creatinine), mineral levels (Ca++ and P) and lipid peroxidation (MDA) and antioxidant Capacity (SOD, CAT and TAC). Histological examination was performed on liver, kidney and lung tissues collected from the rats.

Results: The exposure to EG increased MDA, ALT, AST, ALP, total protein, urea, creatinine, minerals, while reducing antioxidant markers levels, indicating oxidative stress, liver dysfunction, as well as acute renal failure with crystal deposition and primary pulmonary toxicity. Treatment with ethylene glycol and milk thistle extract (G4) resulted in significant improvements, marked by significant increase in the levels of antioxidant enzymes, decreased levels of MDA, ALT, AST, ALP, urea, creatinine, minerals in the serum, and improved histological structure of the liver, kidney, and lung tissues, indicating tissue recovery.

Conclusion: Milk thistle extract relieve ethylene glycol-induced hepatic, renal and pulmonary toxicity, via increasing antioxidants and decreasing lipid peroxidation.

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Key Words: Ethylene glycol toxicity, hepatorenal toxicity, milk thistle extract, pulmonary toxicity.

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INTRODUCTION

Consuming fruits and vegetables daily is recommended to improve immunity and reduce the likelihood of developing chronic illnesses. Producers are using chemical ripen substances such as ethylene gas, acetylene, ethanol, and methyl jasmonate to provide fully grown fruits in to satisfy customer needs[1]. Despite the possible hazards to health, farmers continue to use these ripen compounds to accelerate the maturation of fruits[1,2].

Ethylene glycol (EG) is used to create ethylene gases, which break down complex carbs into simpler sugars, speeding up the maturation process and enhancing fruit appearance[3]. This practice is common in Egypt where ethylene is used to ripen various climacteric fruits and vegetables such as persimmons, bananas, and tomatoes[1]. When ingested, EG disrupts the body's redox and antioxidant balance because of its high levels of water solubility, which allows its faster absorption and dispersion throughout the body[4]. This disruption can result in various harmful effects, such as hepatorenal toxicity, pulmonary system dysfunction, acidosis, and the release of inflammatory enzymes[4,5].

Antioxidants are effective in hindering the formation of free radicals or their propagation, which can decrease oxidative stress and enhance the immune function[6]. Botanical remedies are recognized for their antioxidant and anti-inflammatory characteristics, which can help prevent and defend against diseases and environmental toxins[7].

For centuries, milk thistle, or Silybum marianum, an Asteraceae plant grown in Northern Africa has been utilized to treat a variety of health conditions, like liver, gallbladder, kidney, lung, spleen, prostate, and nervous system disorders[8-10]. Its seeds contain a 26.2 mg/g of silymarin, a compound composed of silibinin, silychristin, silydianin, isosilybin, isosilychristin, polyphenols, and fatty acids[11,12].

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Milk thistle extract contains silymarin, a compound that has antioxidant and anti-inflammatory effects. It can reduce oxidative stress, lipid peroxidation, insulin resistance, mitochondrial dysfunction, and cellular necrosis caused by chemical intoxication[9-11,13]. Additionally, it can inhibit the release of inflammatory cytokines and neutrophil migration, which are associated with inflammation induction[14].

Although milk thistle tea is often used as an herbal remedy for liver ailments[15], its potential to alleviate the toxic effects of ethylene glycol has not been explored. Therefore, this study's goal is to uncover whether milk thistle extract (silymarin) can reduce the oxidative stress and damage to liver, kidney and lung tissues caused by ethylene glycol in male albino rats.

MATERIALS AND METHODS

Chemicals and extract preparation

A solution of ethylene glycol (Sigma–Aldrich) was created by dissolving 3g/ml in distilled water to be given at a dosage of 6 g/kg rat[16]. Milk thistle extract (silymarin) was obtained by infusing 100 g of freshly ground milk thistle seeds obtained from different supplies (target market or market) local market with 1200 ml of boiling water at 100°C for 210 minutes[17]. The extract was then sieved and heated to 40 °C before being dried and stored at -20°C. The extract was administered at dosages equivalent to the human therapeutic dose[18].

Animals and experimental design

The Ethical Research Committee of the Faculty of Science at Damanhour University in Beheira, Egypt, granted ethical permission for the experiment prior to its begin (DMU-SCI-CSRE-22-12-01(2022)). Forty adult male albino rats Rattus norvgicus, 150-180g in weight, were obtained from the Veterinary Science Institute in Cairo, Egypt. The rats were left for two weeks to acclimatize in groups of five each cage in an animal home before the experiment beginning of the experiment, with unlimited water and food available to them. The animal housing provided an environment at 20-25°C and a natural lighting cycle.

The rats were separated into four groups (10 rat/ group):

Group 1- Control: rats served a normal diet and provided with drinking water for 30 days without intervention.

Group 2- Milk Thistle extract (MT): rats were given 151.2 mg/kg/day of milk thistle extract orally for 30 days.

Group 3- Ethylene Glycol (EG): rats received 2 ml of ethylene glycol/kg/day orally for only three days.

Group 4- Ethylene glycol and milk thistle extract (EG + MT) : rats were given 2 ml of ethylene glycol/kg/day orally for the first three days, followed by treatment with 151.2 mg/kg/day of milk thistle extract orally for 1 month.

At the end of the experiment, under sodium pentobarbital anaesthesia, blood was drawn using a Micro Hematocrit Capillaries from the retro-orbital sinus area from overnight fasted rats. After allowing the blood to coagulate, the samples were spun for 15 min at 10,000 g to separate the clear serum samples which were stored at 20°C for later analysis. After cervical dislocation under anesthesia, the rats were immediately dissected for then samples of liver, kidney, and lung tissues were collected.

Liver functions

The levels of alanine aminotransferase enzyme (ALT), aspartate aminotransferase enzyme (AST), and alkaline phosphatase enzyme (ALP) using enzymatic method[19] with Diamond Diagnostics kits (Egypt) (CAT. NO. AL 10 31 (45), CAT. NO. AS 10 61 (45) and CAT. NO. AP 10 20, respectively).

Protein levels

A commercial kit from Diamond, Egypt (CAT. NO. TP 20 20) was used to quantify total protein content as according Henry[20] instructions[20], whereas another kit (CAT. NO. AB 10 10) from the same manufacturer was used to estimate albumin content according to Wotton and Freeman[21].

Kidney functions

A commercial kit from Biodiagnostic, Giza, Egypt (CAT. NO. UR 21 10) was used to measure the amount of urea in accordance with Fawcett and Scott[22] instructions, while a different kit (CAT. No. CR 12 10) from the same supplier was used to determine the quantity of creatinine in accordance with Henry[23] kinetic colorimetric method.

Mineral levels

According to the manufacturer's instructions for Biodiagnostics kits (CAT. NO. CA 12 10 and CAT. No. PH 17 10) for blood levels of mineral including calcium (Ca) and phosphorus (P) were assessed the method described by Gindler and King[24] and El-Merzabani, Anwer-El-Aaser[25], respectively.

Assessing Oxidative Stress: Lipid Peroxidation and Antioxidant Capacity in the blood

Superoxide dismutase enzyme (SOD), catalase enzyme (CAT), and polyunsaturated fatty acids peroxidation marker malondialdehyde (MDA) activity were measured using test kits from Bio-diagnostics, Egypt (CAT. No. SD 25 21, CAT. No. CA 25 17 and CAT. No. MD 25 29), according to the methods described by Claiborne[26], McCord and Fridovich[27] and Draper and Hadley[28], respectively, while kits from Bio-diagnostics, Egypt (CAT. NO. TA 25 13), were applied in accordance with the procedure described by Koracevic, Koracevic[29], to estimate the total antioxidant capacity (TAC).

Histopathological investigation

Tissues from the liver, kidney, and lung were rinsed

quickly with a normal saline solution (0.9% NaCl), fixed in 10% formaldehyde solution for 24 hours, before being dehydrated, and embedded in paraffin, as initially outlined by Suvarna, Layton[30]. Hematoxylin and eosin (H&E) was used to stain tissue slices, and an Olympus CX40 microscope was used to view the sections. NIH ImageJ 1.53t software was used to evaluate (length and diameters, percentage, area etc.).

Statistical Analysis

A statistical software program (SPSS) version 20.0 for windows (SPSS Inc., Chicago, IL, USA) with a significant difference was used to perform a one-way ANOVA followed by a Post Hoc test (Tukey) multiple comparison test to determine differences between groups. The mean value along with the standard deviation was used to express the data.

RESULTS

Effect of ethylene glycol on Mortality percentage

The rats that received ethylene glycol without milk thistle extract treatment had a mortality rate of 30%, while the rats treated with milk thistle extract after ethylene glycol intoxication had a mortality rate of 10%.

Milk thistle extract modulated ethylene glycol-induced disturbance of liver function

(Table 1) shows that there were no significant changes in the levels of serum liver enzymes (ALT, AST, ALP), and protein between the milk thistle extract treated group (G2) and the control group (G1). Nevertheless, compared to the control group (G1), ethylene glycol-intoxicated rats (G3) had significantly higher levels of liver enzymes, and total protein as well as significantly lower levels of albumin ($p < 0.001$). Remarkably, administration of milk thistle extract to ethylene glycol-intoxicated rats (G4) resulted in a significant reduction in liver enzymes, and total protein levels, whereas albumin levels were significantly higher when compared to the ethylene glycol-treated group (G3) ($p < 0.001$).

Milk thistle extract modulated ethylene glycol-induced disturbance of kidney function

In comparison to the control group (G1), rats given milk thistle extract (G2) caused insignificant effect in the levels of urea, calcium, or phosphorus, as depicted in (Table 2). Conversely, a significant reduction ($p < 0.001$) in creatinine levels was detected in the milk thistle extract-treated group (G2) compared to the control group (G1). Ethylene glycol intoxicated rats (G3) exhibited significant elevations ($p < 0.001$) in their serum levels of urea, creatinine, calcium, and phosphorus compared to the control group (G1). However, treatment with milk thistle extract after ethylene glycol intoxication (G4) significantly decreased the markers of kidney function and minerals compared to ethylene glycol-intoxicated rats (G3).

Milk thistle extract inhibited lipid peroxidation mediated by ethylene glycol and improved the antioxidant status

In (Table 3), the mean ± S.D values of lipid peroxidation (MDA) and antioxidant capacity (CAT, SOD, and TAC) are presented. The administration of milk thistle extract (G2) significantly improved the overall antioxidant capacity of rats and reduced MDA levels. However, the intoxication with ethylene glycol (G3) caused a significant elevation in MDA, as well as significant declines in CAT, SOD, and TAC levels compared to the control group of rats (G1) ($p < 0.001$). Nevertheless, rats given milk thistle extract after being intoxicated with ethylene glycol (G4) exhibited a significant elevation in CAT, SOD, and TAC levels and a significant decline in MDA levels when compared to ethylene glycol-treated group of rats (G3) ($p < 0.001$).

Histological examination

Milk thistle extract modulated ethylene glycol-induced hepatotoxicity

Liver tissue analysis was conducted on control group (G1) and milk thistle-treated group (G2) (Figure 1 A-F). The liver tissue showed typical hepatic lobule structure in both groups, with hepatocyte strands extending from the central vein and separated by blood sinusoids (Figure 1 A-F). The polygonal hepatocytes have big basophilic eccentric nuclei and homogeneous eosinophilic cytoplasm (Figure 1 C, F). The margin of the liver lobule contained portal triad elements, which included branch of portal vein, branch of hepatic artery, and bile ductules (Figure 1 B, E). In rats treated with milk thistle extract (G2), there was mild congestion and dilation observed in the portal vein in the liver sections (Figure 1 E).

The liver tissue of rats intoxicated with ethylene glycol (G3) showed severe hepatic injury, including disorganized hepatic cords, congested and dilated central veins, hypertrophic hepatocytes with cytoplasm with vacuoles (Figure 1 G, H, I). The morphometric data analysis showed a significant increase in the area of the central vein and the dimensions of hepatocytes, along with a significant decrease in the dimensions of hepatocyte nuclei, in ethylene glycol treated rats (G3) in comparison to control groups (G1&G2) as indicated in (Figures 2-3).The portal area showed inflammatory cell infiltration, proliferated bile ductules, and congested and dilated portal veins (Figure 1 G, H). However, in rats treated with milk thistle extract after ethylene glycol intoxication (G4), normal hepatic lobular structure with well-defined sinusoids and normal centrilobular hepatocytes were observed (Figure 1J, K, L). Mild hypertrophic periportal hepatocytes, minor cellular infiltration, mild bile ductule proliferation, and mild portal vein dilation and congestion were also present in this group (Figure 1J, K). The morphometric data analysis revealed insignificant increase in central vein area in comparison to milk thistle treated group (G2) along with insignificant increase in hepatocyte length and insignificant decrease in nuclear dimensions in comparison to control groups (G1&G2) (Figures 2-3).

Milk thistle extract modulated ethylene glycol-induced renal toxicity and crystals deposition

The kidney tissue of control groups (G1&G2) displayed the normal structure of renal corpuscles and renal tubules (Figure 4 A-F). The renal corpuscles were found to contain multiple glomerular capillary loops encircled by a limited Bowman's space and capsule. Bowman's capsule bordered with simple squamous epithelium (Figure 4 B, C, E, F). Juxtaglomerular smooth muscle cells were also identified between the vascular pole of the glomerulus and macula densa cells (Figure 4 C, E, F). Proximal convoluted tubules were observed to have a narrow lumen lined with a single layer of long cuboidal cells with brush border microvilli, while distal convoluted tubules had a comparatively larger lumen surrounded by smaller, flattened cubical epithelium. The epithelium of the collecting tubules appeared as short, pale-stained cuboidal cells with a relatively wider lumen than the distal tubules, and the Henle's loop was lined with simple squamous epithelium (Figure 4 B, C,E).

Conversely, the kidney sections of rats (G3) intoxicated with ethylene glycol displayed disrupted cortical architecture with significant distortions in the renal corpuscles and severe dilations of the renal tubules (Figure 4 G). The renal corpuscles exhibited severe damage to the glomeruli, including widening of the Bowman's space, hypertrophic glomerular capillary, congestion, and ruptured Bowman's capsules (Figure 4 H, I). Morphometric analysis revealed significant increase in renal corpuscles, Bowman's space, and glomeruli size compared to control groups (G1&G2) (Figure 5).

The renal tubules showed severe dilation with epithelial vacuolization and nuclear pyknosis, and some tubules even showed ruptured epithelial lining (Figure 4 H, I). Additionally, a significant increase in proximal and distal tubule diameter and collecting duct dimensions in comparison to control groups (G1&G2) were identified (Figure 6). Furthermore, the lumen of the renal tubules contained numerous polymorphic irregular crystals accompanied by hemorrhage (Figure 4 G, H, I).

In the group of rats treated with ethylene glycol and milk thistle extract (G4), kidney sections revealed significant tissue recovery compared to the ethylene glycol-intoxicated group (Figure 4 G-L). The cortical architecture appeared nearly normal with normal renal corpuscles and mildly dilated collecting tubules (Figure 4 K,L). In G4, Renal corpuscles exhibited significant decrease in size compared to ethylene glycol treated group (G3) while exhibited a significant increase in renal corpuscles size compared to G1&G2 (control groups) (Figure 5). However, there were insignificant increases in the size of glomeruli, Bowman's space, and the diameter of proximal and distal convoluted tubules, as well as the length of the collecting duct, compared to control groups (G1&G2) (Figures 5-6). Additionally, the damage to the renal tubules was almost recovered, with limited hemorrhage spots and no crystal deposition observed (Figure 4 K, L).

Milk thistle extract modulated ethylene glycol-induced pulmonary toxicity

The lung tissues of the control group (G1) and milk thistle treated group (G2) were examined, revealing normal lung spongy architecture with bronchioles, clusters of alveoli (alveolar sacs), thin alveolar septa, pulmonary blood vessels, and fibrous tissues (Figure 7 A-F). The bronchioles appeared as thin-walled tubules lined with folded columnar cells and goblet cells and encircled with a thin bronchiolar smooth muscles layer (Figure 7 A, D). Alveoli are lined with alveolar epithelium (pneumocytes) that appeared as flattened or rounded in shape cells separated by thin interalveolar septa (Figure 7 C, F). Nevertheless, compared to the control group of rats (G1), milk thistle-treated rats (G2) exhibited a significant increase in alveolar space (aeration area percentage) (Figure 8).

Conversely, lung tissue examination of rats intoxicated with ethylene glycol (G3) showed remarkable pathological changes. These changes included the presence of severe congestion and edema, severe bronchiole dilation, infiltration of inflammatory cells, disruption of alveolar septa and interstitial cell expansion (Figure 7 G- I). The bronchioles showed collapsed epithelial folds, and in some areas, the bronchiolar epithelium was desquamated, filled with red blood cells and surrounded with thickened peribronchiolar tissue (Figure 7 G). The interalveolar septa were severely thickened, and there were large, irregular emphysema spaces resulting from septal rupture (Figure7I). Morphometric analysis revealed significant increase in interalveolar septa resulting in significant decrease in aeration area compared to control groups (G1&G2) (Figures8-9). Additionally, the pulmonary vessels exhibited severe dilation, congestion, and wall hyperplasia, (Figure 7 H).

On the other hand, examination of lung tissue sections of rats intoxicated with ethylene glycol and treated with milk thistle extract (G4) revealed a significant restoration of normal lung morphology (Figure 7 J-L). The sections showed normal bronchiolar and alveolar architecture with mild interalveolar septa thickening in some areas, mild pulmonary vessels congestion, and mild cellular infiltration (Figure 7 J-L). The bronchioles showed a lining of folded epithelial cells that bordered by a thin layer of smooth muscle cells, with mild peribronchial infiltrated cells (Figure 7 J). Additionally, slightly widened alveoli with a thin layer of pulmonary cells were also observed (Figure7L). The interalveolar septa showed significant decrease in their thickness if compared to ethylene glycol treated group (G3) resulting in significant increase in aeration area percentage if compared to ethylene glycol treated group (G3) (Figure8-9).

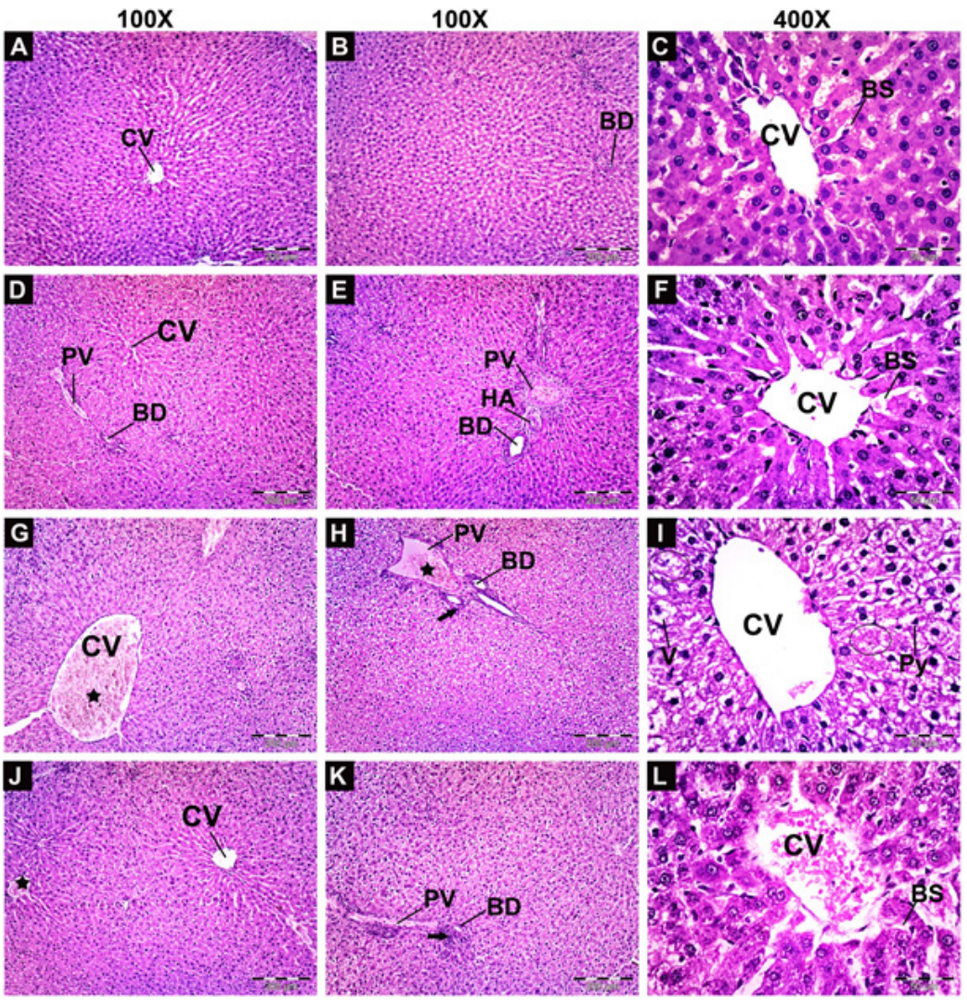

Fig. 1: Liver sections of different experimental groups. Liver sections of control group (G1) (A-C), milk thistle treated group (G2) (D-F) showing normal liver architecture including central vein (CV), blood sinusoids (BS),bile ductules (BD), hepatic artery (HA) and portal vein (PV). G-I: liver sections of ethylene glycol treated group (G3) showing hepatic cords disorganization, blood vessels congestion (*), dilated central vein (CV), dilated portal vein (PV), cellular infiltration (black arrows), vacuolated hepatocytes (V) with pyknotic nuclei (Py) and ill-defined blood sinusoids, cellular necrosis marked by black circle. J-L: liver sections of ethylene glycol and milk thistle treated group (G4) showing normal hepatic cord separated by blood sinusoids (BS), few congested blood vessels (*),infiltrated cells (black arrow). H&E

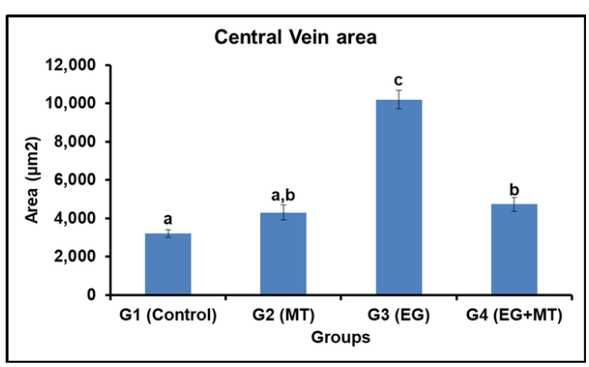

Fig. 2: Morphometric analysis of central vein area (µm2) in liver sections of different experimental groups. Ethylene glycol-administration (G3) caused significant increase in central vein area compared to control groups (G1&G2), while ethylene glycol rats treated with milk thistle group (G4) displayed significant decrease in central vein area compared to G3. *Data are means ± SD (standard deviation). Bars with distinct superscript letters indicate statistically significant differences at $p < 0.05$.

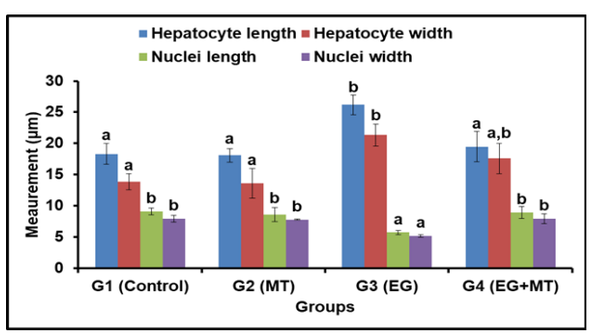

Fig. 3: Morphometric analysis of hepatocyte and nuclear dimensions (µm) in liver sections of different experimental groups. Ethylene glycol-administration (G3) significantly increases hepatocyte dimensions and significantly decreases in nuclear dimensions compared to control groups (G1&G2). Ethylene glycol and milk thistle treated rats (G4) showed insignificant increase in hepatocyte length and insignificant decrease in nuclear dimensions compared to control groups (G1&G2). *Data are means ± SD (standard deviation). Bars with distinct superscript letters indicate statistically significant differences at $p < 0.05$.

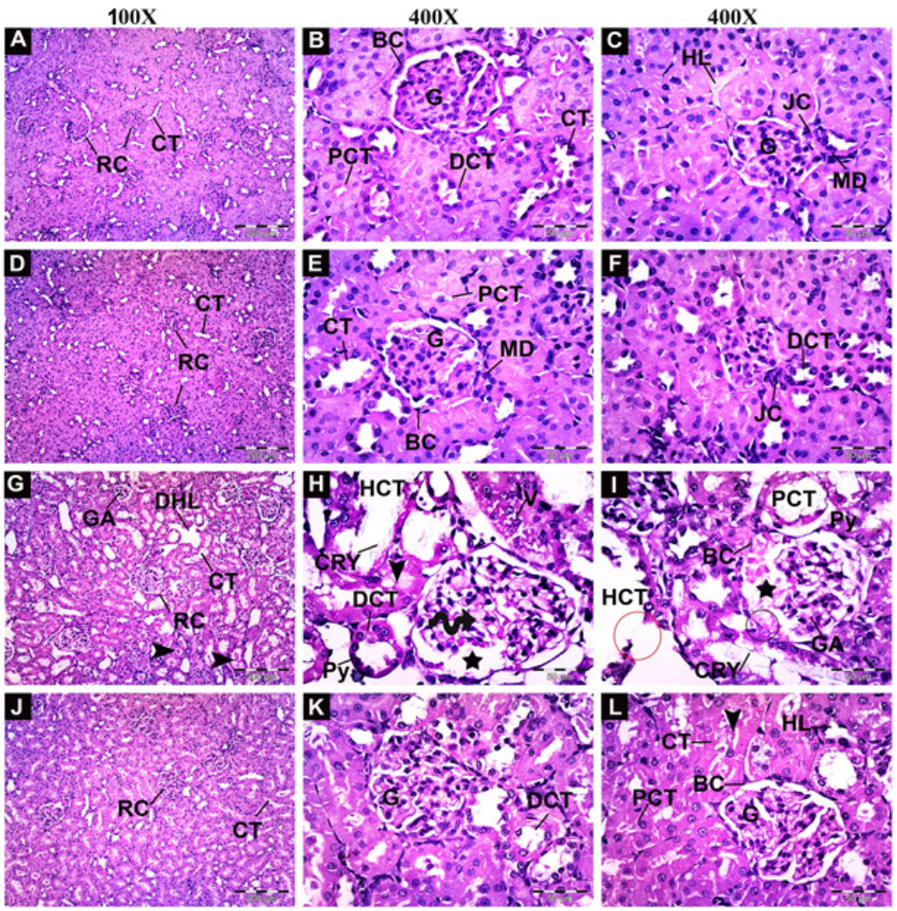

Fig. 4: Kidney sections of different experimental groups. Kidney sections of control group (G1) (A-C), and milk thistle treated group (G2) (D-F) showing normal kidney structure with renal corpuscles (RC), glomerular capillaries (G) surrounded by bowman's capsule (BC). Notice, juxtaglomerular cells (JC), macula densa cells (MD), proximal convoluted tubule (PCT), distal convoluted tubule (DCT), collecting tubules (CT), and Henle's loop (HL). G-I: kidney sections of ethylene glycol treated groups (G3), notice few atrophic glomeruli (AG), dilated Henle's loop (DHL), hypertrophic glomeruli (curled arrow), dilated congested bowman's space (*),ruptured epithelial lining of Bowman's capsule (black circle), hypertrophic collecting tubules (HCT) with ruptured epithelial lining (red circle), and precipitated crystals (CRY) causing hemorrhage (head arrows). J-L: kidney sections of ethylene glycol and milk thistle treated group (G4) showing nearly normal renal corpuscle (RC) structure with regular renal tubules lining, except for some areas of hemorrhage in collecting tubule lumen (head arrow). H&E

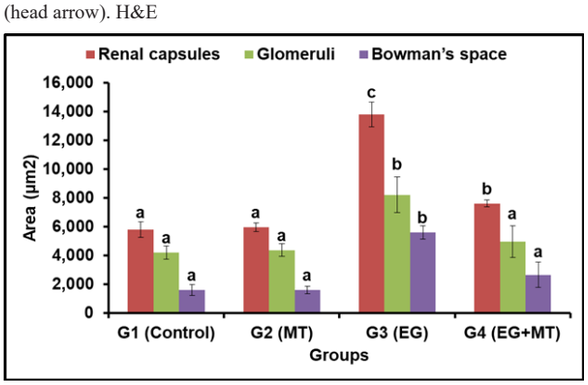

Fig. 5: Morphometric analysis of renal capsules, glomeruli, and Bowman's space areas (µm2) in kidney sections of different experimental groups. Ethylene glycol administration (G3) caused significant increase in renal capsules, glomeruli, and Bowman's space size compared to control groups (G1&G2), while ethylene glycol and milk thistle extract-treated group (G4) showed significant decrease in renal capsules size compared to G3 and insignificant increase in glomeruli, and Bowman's space size compared to control groups (G1&G2).*Data are means ± SD (standard deviation). Bars with distinct superscript letters indicate statistically significant differences at $p < 0.05$.

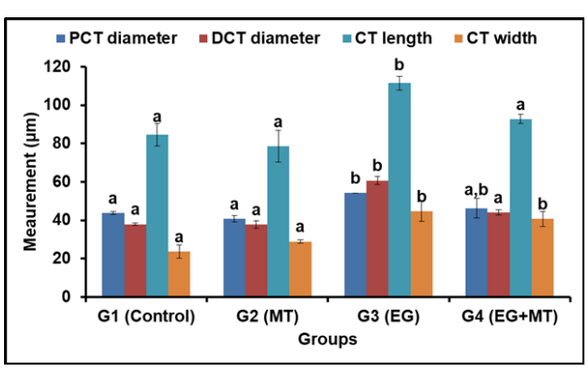

Fig. 6: Morphometric analysis of renal tubules dimensions (µm) in kidney sections of different experimental groups. Ethylene glycol administration (G3) caused significant increase in renal tubules dimensions compared to control groups (G1&G2), while ethylene glycol and milk thistle extract-treated group (G4) showed insignificant increase in diameter of proximal and distal convoluted tubules (PCT&DCT) compared to control groups (G1&G2) and insignificant increase in collecting duct (CT) length compared to control groups (G1&G2). *Data are means ± SD (standard deviation). Bars with distinct superscript letters indicate statistically significant differences at $p < 0.05$.

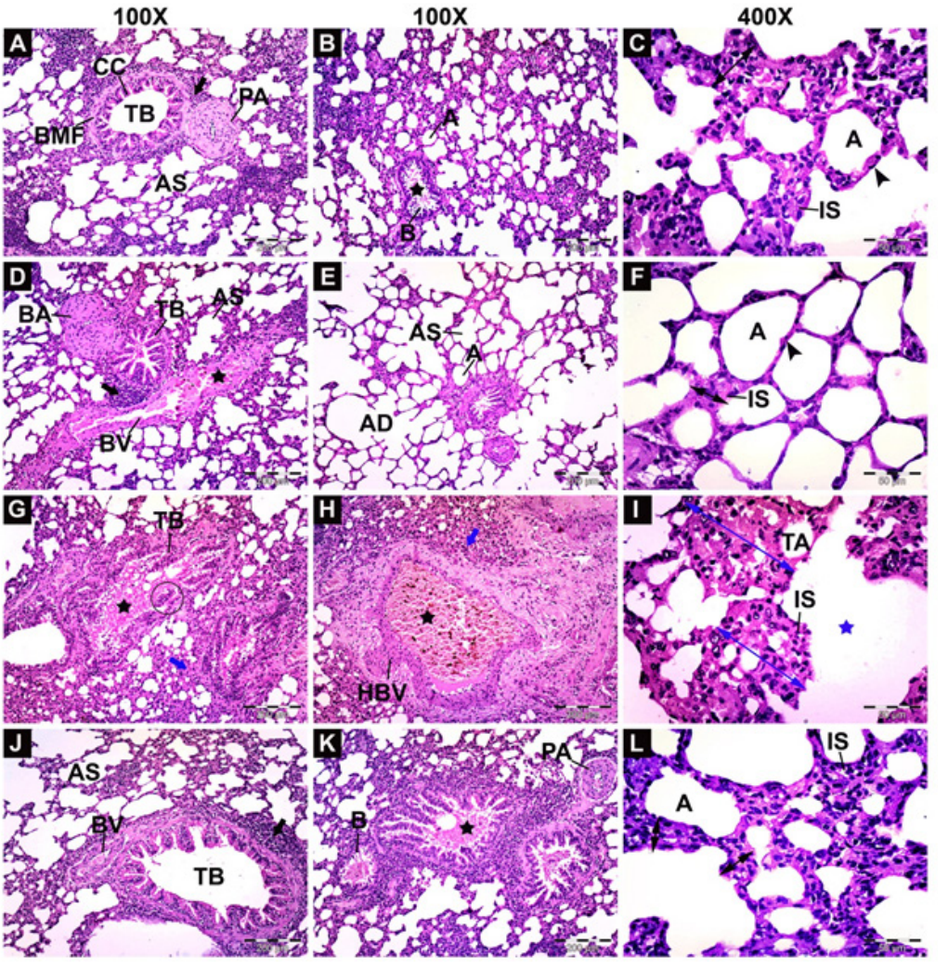

Fig. 7: Lung sections of different experimental groups. Control group (G1) (A-C). and milk thistle treated group (G2) (D-F). Showing normal lung morphology which consist terminal bronchiole (TB) with columnar cells (CC) lining and surrounded by bronchiole muscles fibers (BMF), peribronchiolar tissue (black arrow), bronchiole (B), blood vessel (BV), alveolar sacs (AS), alveoli (A), air duct (AD), pulmonary artery (PA), interalveolar septa (IS) and pneumocytes (head arrow). G-I: Lung sections of ethylene glycol treated group (G3) notice, congestion of terminal bronchiole and blood vessels (*), desquamation of bronchiole epithelia (black circle) , hypertrophic blood vessels (HBV), interstitial cells hyperplasia (blue arrow), thick interalveolar septa (blue double headed arrow), trophic alveoli (TA), emphysema (blue asterisk).J-L: Lung section of ethylene glycol and milk thistle treated group (G4) showing almost normal lung tissue morphology, with wise alveolar space (AS), thin interalveolar sepata (IS).H&E

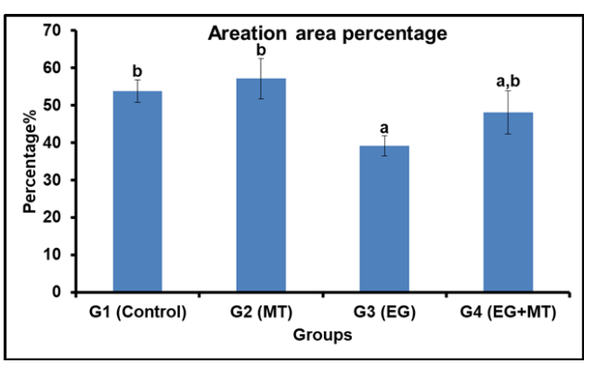

Fig. 8: Morphometric analysis of aeration area percentage (%) in lung sections of different experimental groups. Milk thistle treatment (G2) significantly increases aeration area in comparison to control group (G1), while ethylene glycol treatment (G3) caused significant decrease in aeration area compared to control groups (G1&G2). However, rats treated with ethylene glycol and milk thistle (G4) showed insignificant decrease in aeration area in comparison to control groups (G1&G2). *Data are means ± SD (standard deviation). Bars with distinct superscript letters indicate statistically significant differences at $p < 0.05$.

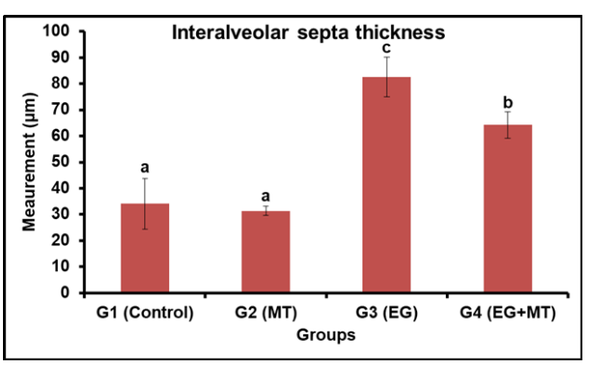

Fig. 9: Morphometric analysis of interalveolar septa thickness (µm) in lung sections of different experimental groups. The administration of ethylene glycol (G3) significantly increases in interalveolar septa thickness compared to control groups (G1&G2). However, ethylene glycol rats treated with milk thistle (G4) showed significant decrease in interalveolar septa thickness in comparison to G3. *Data are means ± SD (standard deviation). Bars with distinct superscript letters indicate statistically significant differences at $p < 0.05$.

Table 1: Serum Biomarkers for Liver Function Assessment

	ALT (U/L)	AST(U/L)	ALP(U/L)	Total protein (mg/dL)	Albumin (mg/dL)
G1 (Control)	36.07±1.10 [a]	113.3±1.53 [a]	195.9±1.21 [b]	5.355±0.025 [a]	4.18±0.1 [c]
G2 (MT)	34.7±0.44 [a]	116.7±1.53 [a]	164.2±1.04 [a]	5.47±0.15 [a,b]	4.02±0.13 [c]
G3 (EG)	54.65±0.65 [c]	177.2±1.04 [c]	982.3±1.55 [d]	7.175±0.055 [c]	3.15±0.05 [a]
G4 (EG+MT)	41.5±0.5 [b]	151.2±1.06 [b]	357.2±2.02 [c]	5.65±0.05 [b]	3.63±0.07[b]
Reference range	Up to 45U/L[31].	Up to 40U/L[31].	200-1000U/L[32]	6.5-8.7mg/dL[33]	3.5-5.5mg/dL[34]

*Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase, ALP; alkaline phosphatase, MT; milk thistle, EG; ethylene glycol, U/L (unit per liter) mg/dL (milligrams per deciliter).

*Data are means ± SD (standard deviation). Means in the same column with different superscript letters are statistically significant ($P < 0.001$) using one-way ANOVA.

Table 2: Serum Biomarkers for kidney Function Assessment

	Urea (mg/dL)	Creatinine (mg/dL)	Ca^{++} (mg/dL)	*P* (mg/dL)
G1 (Control)	28.15±0.35[a]	0.55±0.04[b]	6.61±0.31[a]	5.38±0.54[a]
G2 (MT)	26.15±0.55[a]	0.46±0.035[a]	6.98±0.16[a]	6.1±0.53[a]
G3 (EG)	115.1±1.7[c]	1.27±0.021[d]	13.05±0.07[c]	7.35±0.25[b]
G4 (EG+MT)	42.05±1.05[b]	0.67±0.015[c]	8.9±0.35[b]	5.86±0.06[a]
Reference range	15-50 mg/dL[35]	0.46-3.93 mg/dL[36]	8.1-10.4mg/dL[37]	2.5-5 mg /dL[38]

*Abbreviations: Ca++, calcium; *P*, phosphorus, MT; milk thistle, EG; ethylene glycol, mg/dL (milligrams per deciliter).

*Data are means ± SD (standard deviation). Means in the same column with different superscript letters are statistically significant ($P < 0.001$) using one-way ANOVA.

Table 3: Serum Biomarkers for Lipid Peroxidation and Antioxidant Enzymes

	MDA (nmol/ml)	TAC (mM/L)	SOD (U/ml)	CAT (U/L)
G1 (Control)	12.52±0.2[a]	4.22±0.10[c]	19.25±0.55[c]	38.25±0.65[d]
G2 (MT)	14.62±0.3[b]	4.12±0.11[c]	20.7±0.6[d]	36.15±0.25[c]
G3 (EG)	26.23±0.11[d]	1.68±0.18[a]	7.38±0.28[a]	13.1±0.6[a]
G4 (EG+MT)	19.35±0.25[c]	3.43±0.20[b]	13.42±0.2[b]	28.65±1.15[b]
Reference range	0.9-1.8 µmol/L[39]	0.5 - 2 mM/L[40]	4.7-166 U/mL[41]	0.29-0.41 U/mL[42]

*Abbreviations: MDA; malondialdehyde, TAC; Total antioxidant capacity, SOD Superoxide dismutase enzyme, CAT; catalase, MT; milk thistle, EG; ethylene glycol, nmol/ ml (nanomole/milliliter) , mM/l (millimolar per liter), U/ml (unite per milliliter) and U/L (unit/ liter).

*Data are means ± SD (standard deviation). Means in the same column with different superscript letters are statistically significant ($P < 0.001$) using one-way ANOVA.

DISCUSSION

The utilization of ethylene glycol (EG) as a food ripening agent has been increasing due to the growing demand for off-season products, even though ingestion of this substance can lead to toxicity in multiple organs[2,42] which highlights the need for investigating natural products that can reverse its toxicity. Milk thistle extract (silymarin) has antioxidant, free radical scavenger, and anti-inflammatory properties that could potentially mitigate the harmful effects of chemical toxins[7,14].

Previous studies have indicated that the consumption of ethylene glycol leads to inflammation and oxidative damage due to the generation of free radicals which promotes oxidative degradation of lipids and reduces the body's antioxidant defense mechanism[4,5,43].

In this study, ethylene glycol intoxication (G3) resulted in a significant elevation in MDA levels, which is a marker of lipid peroxidation. This elevation was paralleled with a significant decline in the levels of SOD, CAT, and TAC, indicating a compromised antioxidant defense mechanism in scavenging free radicals. As a result, the liver, kidney, and lung tissues were subjected to pathological damage caused by these free radicals. These results agree with those stated by El Menyiy, Al Waili[5], who demonstrated that exposure to ethylene glycol leads to oxidative damage as verified by rise in MDA levels and decline in SOD and CAT levels. The decrease in levels of SOD and CAT can cause an accumulation of free radicals, resulting in lipid peroxidation, increased cell membrane permeability, complete cell disintegration, and the release of cellular components into the bloodstream[14,44].

The current study revealed that intoxication with ethylene glycol (G3) caused oxidative damage to hepatocytes, resulting in loss of membrane integrity, hypertrophic and necrotic hepatocytes, and elevated blood levels of ALT, AST, ALP, albumin, and total protein. These results are in line with earlier researches that reported similar effects of ethylene glycol on hepatocytes, such as ballooning appearance, vacuolated cytoplasm, necrosis, and increased levels of hepatic enzymes and proteins in the bloodstream[2,9,45]. Additionally, ethylene glycol toxicity induced bile duct hyperplasia and ALP accumulation in the blood, which is attributed to oxidative damage to hepatocytes and impaired biliary system function leading to reduced bile secretion[46]. Similarly, diclofenac intoxication can cause the proliferation of biliary ducts and increase in plasma ALP and bilirubin levels[47].

The current study found that the liver tissues of rats exposed to ethylene glycol (G3) exhibited blood vessel congestion, which matching with the results of previous research after treatment experimental animals with ethylene glycol and caused histopathological changes in liver tissue[4]. The earlier study reported that ethylene glycol exposure led to changes in liver morphology, including cytoplasmic vacuolization and necrosis of hepatocytes, as well as blood vessel congestion[4].

The damaging effects of ethylene glycol on the kidneys, which frequently lead to renal failure and acidosis, have been documented in several investigations. Hepatocytes change ethylene glycol into oxalic acid, which results in calcium oxalate crystal development in the kidney tubules and eventually renal failure[16]. Moreover, the presence of high levels of MDA can stimulate the formation of ROS in renal cells, leading to renal cell necrosis and the release of kidney enzymes into the bloodstream[47,48].

In the current research, ethylene glycol intoxication in rats (G3) caused several forms of renal damage, including Bowman capsule enlargement, glomerular atrophy, renal epithelium necrosis, cytoplasmic vacuolization, and interstitial inflammation, as well as elevated levels of urea, creatinine, and proteins in the serum. These findings are consistent with previous researches, that reported similar outcomes of renal corpuscle widening, glomerular degeneration, and cellular infiltration in rats treated with ethylene glycol[2,5]. Urea, creatinine, and proteins can accumulate in the bloodstream as a result of ethylene glycol-induced renal failure, along with accompanying problems like acidosis and changes in electrolytes[5].

In the present study ethylene glycol toxicity (G3) caused renal damage through multiple mechanisms, including changes in the concentrations of antioxidant enzymes, and inflammatory reactions Husseiny, Farag [49]. This is along with increased serum levels of calcium and phosphorus, which can precipitate as crystals in the renal tubules, leading to widening, and necrosis of the tubular epithelium, edema, and inflammation. Earlier studies have reported similar outcomes.

Previous studies have shown that ethylene glycol intoxication causes elevated serum levels of calcium and phosphorus, which can lead to failure in the renal tubules by formation of calcium phosphate and calcium oxalate crystals, resulting in widening of the proximal convoluted tubules, tubular epithelium necrosis, edema, and interstitial inflammation[43,45,49].

Ethylene glycol causes oxidative damage that leads to vascular dysfunction, characterized by hyperplasia of the pulmonary vessels, congestion, and leakage of red blood cells. This damage affects the blood vessels' epithelium. Previous studies have reported similar outcomes, indicating that oxidative stress caused by ethylene glycol can lead to pulmonary toxicity and vessel congestion[2,42,44,50].

This study demonstrated that ethylene glycol administration (G3) caused significant lung damage, including loss of normal architecture, emphysema, and interalveolar septal thickening and rupture bronchiolar desquamation, and proliferation of fibrous tissue, leading to inflammation and edema. These findings are consistent with previous reports of ethylene glycol-induced respiratory abnormalities. Similar pulmonary dysfunction has been observed in rats treated with nicotine and amiodarone[50,51]. This observation is consistent with prior findings on ethylene glycol's impact on lung tissue[2,42]. Somade, Ajayi[44] observed similar respiratory anomalies in rats exposed to ethylene glycol monomethyl ether, including respiratory distress, alveoli inflammation, and vessel congestion and hyperplasia.

Milk thistle extract (silymarin) has antioxidant and anti-inflammatory properties[7,8,42,52]. In the current study, rats treated with both ethylene glycol and milk thistle extract (G4) had restored redox balance, as evidenced by lower MDA and higher SOD, CAT, and TAC levels when compared to ethylene glycol-only treated rat. These results suggest that milk thistle extract's antioxidant and anti-inflammatory properties may have played a role in reversing ethylene glycol-induced oxidative damage[53].

In the present study, milk thistle extract improved liver and kidney function in ethylene glycol treated rats (G4), as well as recorded decrease in levels of ALT, AST, ALP, proteins, and albumin in the serum. This confirms its ability to reduce lipid peroxidation, minimize cell necrosis and enzyme leakage. Previous studies also showed milk thistle extract's hepatorenal protective effects against lead acetate, carbon tetrachloride, and sepsis-induced oxidative damage through normalization of liver and kidney enzymes and proteins serum levels[9,11,52]. Earlier research has demonstrated that milk thistle extract (silymarin) can protect against oxidative damage in animal models treated with paraquat[54] and busulfan[8] by decreasing lipid peroxidation (MDA), enhancing antioxidant defense mechanisms (SOD and CAT), and improving liver and kidney parameters.

In the same study, milk thistle extracts reduced serum calcium and phosphorus levels, preventing renal tubule

damage, crystal formation and hyperproteinemia in ethylene glycol-treated rats (G4). The extract also improved glomerular morphology, reduced tubular congestion, and restored kidney interstitial tissue. Its antioxidant enzymes and anti-inflammatory reactions may have prevented kidney dysfunction, consistent with previous studies[5,53].

The results of the study reveal that treatment of milk thistle extract has a possible protective effect on lung tissue against ethylene glycol-induced damage. The treated rats (G4) showed improved lung morphology with regular bronchiolar structure, decreased septal thickness and cellular infiltration. These findings align with previous researches that demonstrate the protective effects of milk thistle extract on lung tissue against various toxic agents, including hydrochloric acid[14], mercuric chloride[13], titanium dioxide nanoparticles[56] and paraquat[57]. It is hypothesized that milk thistle extract (silymarin), is believed to involve various molecular and cellular pathways that regulate lung tissue remodeling, bronchial muscle relaxation, and alveolar expansion resulting in increasing bronchial muscle and alveolar size, improving gas exchange and the delivery of oxygen from the blood to the tissues in the lungs[58]. These investigations found that milk thistle extract can decrease inflammatory cell infiltration, peribronchial inflammation, and septal widening in the lungs.

CONCLUSION

Milk thistle extracts reversed ethylene glycol's damaging effects on liver, kidney, lung, and serum parameters in experimental rats. The extract's antioxidant property restored redox balance and reduced cellular damage. These results point to the effect of milk thistle extract as a non-pharmacological treatment and preventative measure for ethylene glycol poisoning. This work offers vital insights into the possible advantages of milk thistle extract as a protective agent against harmful chemicals, while more research is required to discover whether these findings are pertinent to human health.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تقييم كفاءة حليب الشوك (السلبين المريمى) ضد التأثيرات الضارة للايثلين جليكول فى ذكور الجرذان البيضاء

امل ابراهيم بركات، ندى شعبان بدر

قسم علم الحيوان - كلية العلوم - جامعة دمنهور

المقدمة: يُعد الإيثيلين جلايكول مادة مستخدمة بشكل شائع كعامل إنضاج للفواكه، ولكنه قد يتسبب في حدوث ضرر تأكسدي للأنسجة، مما يؤدي إلى فشل وظيفي في الأعضاء. في المقابل، يُعرف نبات السلبين المريمي (الحليب الشوكي) باستخداماته الطبية التقليدية بقدرته على التخلص من الجذور الحرة (الفريراديكال) الضارة وتنظيم الاستجابات الالتهابية.
هدف الدراسة: تهدف هذه الدراسة إلى تقييم فعالية المستخلص المائي لنبات الحليب الشوكي في التخفيف من التأثيرات الضارة للإيثيلين جلايكول على أعضاء ذكور الجرذان البيضاء.
المواد والطرق: تم تقسيم أربعين جرذاً إلى أربع مجموعات: المجموعة الضابطة (G١)، مجموعة المعالجة بمستخلص الحليب الشوكي (G٢) التي تلقت ١٥١,٢ ملجم/كجم يوميًا من المستخلص لمدة شهر، المجموعة الثالثة (G٣) التي تلقت الإيثيلين جلايكول للحث على السمية (٢ مل/كجم/يوم لمدة ٣ أيام)، والمجموعة الرابعة (G٤) التي تلقت كلاً من الإيثيلين جلايكول ومستخلص الحليب الشوكي. شملت التحاليل الكيميائية الحيوية عينات الدم لتقييم وظائف الكبد (ألانين أمينوترانسفراز (ALT)، أسبارتات أمينوترانسفراز (AST)، الفوسفاتاز القلوي (ALP)، بالإضافة إلى البروتين الكلي، الألبومين، وظائف الكلى (اليوريا، الكرياتينين)، مستويات المعادن (الكالسيوم والفوسفور)، قياسات التحلل التأكسدي لليبيدات (المالونديالديهايد(MDA))، ومستويات مضادات الأكسدة (إنزيم سوبر أوكسيد ديسميوتاز(SOD) وكاتاليز (CAT)و القدرة الإجمالية لمضادات الأكسدة (TAC)). كما تم إجراء الفحص النسيجي لأنسجة الكبد، الكلى، والرئتين.
النتائج: أدى التعرض للإيثيلين جلايكول إلى زيادة ملحوظة في مستويات المالونديالديهايد، و ألانين أمينوترانسفراز ، و أسبارتات أمينوترانسفراز ، و الفوسفاتاز القلوي ، والبروتين الكلي، واليوريا، والكرياتينين، ومعادن الدم، مصحوبة بانخفاض في مستويات مضادات الأكسدة، مما يشير إلى إجهاد تأكسدي وخلل في وظائف الكبد والكلى مع ترسب البلورات بالكلى، بالإضافة إلى تسمم رئوي أولي. أما في المجموعة المعالجة بالإيثيلين جلايكول ومستخلص الحليب الشوكي، فقد لوحظت تحسنات ملحوظة تمثلت في زيادة نشاط الإنزيمات المضادة للأكسدة، وانخفاض مستويات المالونديالديهايد، و ألانين أمينوترانسفراز ، و أسبارتات أمينوترانسفراز ، و الفوسفاتاز القلوي ، واليوريا، والكرياتينين، والمعادن، إضافة إلى تحسن في الهيكل النسيجي للكبد، الكلى، والرئتين، مما يدل على تعافي الأنسجة.
الاستنتاج: يُظهر مستخلص الحليب الشوكي فعالية في التخفيف من السمية الكبدية، الكلوية، والرئوية الناتجة عن الإيثيلين جلايكول من خلال تعزيز نشاط مضادات الأكسدة وتقليل مستويات التحلل التأكسدي لليبيدات (المالونديالديهايد).