International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 10s, 2025
https://theaspd.com/index.php

The Protective Role Of Silymarin Against The Effects Of Dioxin On Some Physiological And Histological Parameters In Female Albino Rats And Their Fetuses

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Abstract:
This study aimed to evaluate the protective role of the plant-derived compound silymarin, extracted from Silybum marianum, against the toxic physiological and histological effects of dioxin in pregnant female albino rats and their fetuses. The experiment was conducted in the animal house of the College of Veterinary Medicine, University of Tikrit, under controlled laboratory conditions with adequate food and water provided. A total of 24 female albino rats, aged between 18–20 weeks and weighing between 200–250 grams, were randomly divided into five groups, with six rats in each group: Group 1 (Control): Healthy females administered distilled water daily for 30 days. Group 2: Treated with a low dose of dioxin at 4 µg/kg body weight. Group 3: Treated with a high dose of dioxin at 40 µg/kg body weight. Group 4: Treated with a low dose of dioxin (4 µg/kg) combined with silymarin. Group 5: Treated with a high dose of dioxin (40 µg/kg) combined with silymarin. The treatment period lasted for 30 days. After this period, three females from each group were allowed to mate with healthy males, while the other three females were sampled. Blood samples were collected from the heart and organs with dioxin and silymarin exposure, especially the liver, were harvested for analysis. After marriage, the presence of a cervical plug was considered a sign of early pregnancy. Pregnant women were sampled the day before parturition for blood and placental collection.
Keywords: *Dioxin, Silymarin, Offspring*

INTRODUCTION

Pollutants are responsible for many of today's ecological problems, affecting ecosystems and ecosystem health. Dioxin is considered one of the most toxic chemicals in animals due to its high toxicity, as it can form and accumulate in animal tissues, which can affect the reproductive health and function of mammals. The pathogen is produced by uncontrolled combustion processes and enters the food chain through air, water and soil, posing a serious threat to living organisms, especially pregnant women and their babies (Al-Shaibi et al., 2023).Dioxin is an umbrella term for a group of chemicals known for their toxicity to living organisms and their effects on living organisms. Dioxin is classified as a persistent organic pollutant (POP) due to its long-term presence in the environment and biodegradability. Among the toxic and well-characterized chemicals is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is considered one of the most toxic chemicals (Holsapple et al., 2019 ; Ralph et al., 2019).Dioxin toxicity is associated with increased oxidative stress, mainly through the activation of aryl hydrocarbon receptors (AhRs). This process leads to the formation of reactive oxygen species (ROS), which leads to the damage and destruction of immune cells, including enzymes such as superoxide dismutase (SOD) and catalase (CAT). Oxidative stress plays an important role in tissue damage, especially to the reproductive organs, leading to several obstetric complications (Huang et al., 2021).Air pollution is one of the major environmental hazards and causes of reproductive impairment (Huang et al., 2021). Exposure to dioxins

damages the reproductive system, as oxidative stress impairs their function, which can affect fertility and reproductive health (Ralph et al., 2019). Furthermore, dioxin exposure during pregnancy can affect fetal development, leading to fetal growth retardation, birth defects, increased risk of miscarriage, and preterm birth (Yu et al., 2016 ; Li et al., 2019).On the other hand, functional foods containing antioxidants have emerged as a promising strategy to mitigate the negative effects of environmental pollutants. Among these foods, milk thistle (Silybum marianum) is known for its phenolic compounds, such as punicalagin and ellagic acid, with silymarin being the main constituent. Silymarin, a polyphenolic compound, has been shown to have hepatoprotective effects and is considered one of the compounds that protect the liver from oxidative damage, including oxidative damage caused by dioxins.In addition to its anti-inflammatory properties, silymarin exhibits anti-inflammatory properties and can strengthen immune function, helping to fight oxidative stress. It also improves cell function, protects against DNA damage, enhances the expression of antioxidant enzymes, and reduces oxidative stress in the liver and kidney (Ketor et al., 2002 ; Abénavoli et al., 2010 ; Polyak et al., 2010 ; Faria et al., 2022).

MATERIALS AND METHODS

This study used 24 female albino mice obtained from the Animal Breeding Center of the College of Veterinary Medicine, Tikrit University. The mice were 18–20 weeks old and weighed 200–250 grams. They were housed and acclimatized for 10 days under standard laboratory conditions. The animals were kept in plastic cages covered with wire mesh, and the cage floors were lined with clean sawdust, which was changed periodically to maintain hygiene, according to standard laboratory protocols. The cages were maintained in a room with controlled conditions, kept at a temperature of 22 ± 3°C, with adequate ventilation.The rats were fed a commercial standard diet consisting of 35% wheat, 34% yellow corn, 20% soybean, 10% animal protein, and 1% powdered milk, supplemented with 50 grams of preservatives, vitamins, and antifungal agents, as described by Abel *et al.* (1983). Water was provided in plastic containers securely attached to the cages to prevent spillage.

Animal Dissection (Anatomy of Animals): At the end of the experiment, the rats were fasted for 24 hours before dissection. They were then anesthetized using chloroform, allowed approximately three minutes for the anesthetic to take full effect, and blood was drawn directly from the heart via cardiac puncture. Blood samples were collected into plain plastic tubes without anticoagulant and left at an inclined position for 30 minutes to allow clotting. The samples were then centrifuged at 3000 rpm for 15 minutes to separate the serum. The serum was carefully collected using a micropipette and stored in clean tubes at –20°C until biochemical analysis.Following blood collection, the animals were dissected by opening the abdominal cavity to remove the required organs (mainly the kidneys). Surrounding connective tissues and fat were removed, and the organs were washed with water. The kidneys, liver, lungs, and aorta were then fixed in formalin for 24 hours. After fixation, the tissues were washed with tap water and preserved in 70% alcohol for further processing, according to the method described by Al-Haj (1998).

Experimental Design: This study involved the use of dioxin and silymarin, and included 24 female rats divided into five groups, with six animals in each group, over a period of four weeks, as follows:

Stage One- Treatment: -

Group 1 (Control Group): Healthy females administered distilled water orally for 30 days.
Group 2: Treated with low-dose dioxin at 4 µg/kg body weight.
Group 3: Treated with high-dose dioxin at 40 µg/kg body weight.
Group 4: Treated with low-dose dioxin (4 µg/kg) combined with silymarin.
Group 5: Treated with high-dose dioxin (40 µg/kg) combined with silymarin.

The oral administration continued for 30 days. After this period, three animals from each group were sacrificed for physiological and histological analysis, while the remaining animals were used for mating with healthy males

to investigate whether the toxic effects of dioxin could be transmitted to the fetuses through exposure during the gestational period.

Stage Two- Mating: - After the 30-day treatment period, mating was carried out as follows:

Mating of control group females (given distilled water) with healthy males.

Mating of females treated with low-dose dioxin.

Mating of females treated with high-dose dioxin.

Mating of females treated with low-dose dioxin and silymarin.

Mating of females treated with high-dose dioxin and silymarin.

Mating Procedure: Healthy male rats, aged 10–12 weeks and weighing between 250–300 grams, were selected and housed overnight in plastic cages with females of the same strain. The following morning, the females were examined for signs of mating, indicated by the presence of a vaginal plug, sperm in the vaginal smear, blood at the vaginal opening, or vaginal enlargement were taken as indicators of successful mating. The day on which these signs were observed was considered day 0 of gestation (Fox *et al.*, 2006).

RESULTS

Oxidative Balance in Female Rats: The results of this study revealed a significant increase ($P \leq 0.05$) in oxidative stress markers and a significant decrease ($P \leq 0.05$) in antioxidant levels. Furthermore, there was a notable elevation in DNA oxidative stress indicators in the serum of female albino rats treated with various doses of dioxin and silymarin compared to the control group.

Oxidative Products: The findings demonstrated a significant rise in oxidative stress markers, including isoprostanes, malondialdehyde (MDA), and 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a key indicator of oxidative DNA damage. These markers showed a statistically significant increase ($P \leq 0.05$) in all treated groups relative to the control group, as shown in Table.(1)

Table (1):** Levels of oxidative stress products (Isoprostanes, Malondialdehyde [MDA], and 8-OHdG) in the serum of female albino rats.

Groups	Control	Low-Dose Dioxin	High-Dose Dioxin	Low-Dose Dioxin with Silymarin	High-Dose Dioxin with Silymarin
Isoprostane-8 (µ/L)	24.13±4.43	43.14±2.52	42.69±1.80	30.89±1.31	30.49 ±1.67
MDA (µ/L)	20.59±1.42	93.76±3.98	109.14±8.79	87.04±2.68	85.95±1.86
8-OHdG (µ/L)	56.3±4.97	280.2±27.0	403.7±46.5	194.5±3.91	271.8±48.6

Antioxidant Markers: The current study also revealed a significant decrease ($P \leq 0.05$) in antioxidant enzymes within the serum of treated groups compared to the control group. The antioxidant parameters assessed included Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx). These enzyme levels were markedly lower in the dioxin- and silymarin-treated groups, indicating compromised antioxidant defense mechanisms under oxidative stress conditions, as shown in Table (2).

Table (2): Levels of antioxidant enzymes (SOD, CAT, GPx) in the serum of female albino rats.

Groups	Control	Low-Dose Dioxin	High-Dose Dioxin	Low-Dose Dioxin with Silymarin	High-Dose Dioxin with Silymarin
SOD (µ/L)	6.55±0.09	1.525±0.32	1.34±0.165	1.205±0.024	1.134±0.121
CAT (µ/L)	5.344±0.505	0.677±0.209	0.56±0.04	1.573±0.003	1.549±0.035
GPx (µ/L)	90.22±2.59	4.23±2.51	12.36±1.019	21.914±0.092	23.6±2.82

Liver Enzymes in Female Albino Rats: The study presented a significant increase ($P \leq 0.05$) in the levels of liver enzymes AST (Aspartate Aminotransferase) and ALT (Alanine Aminotransferase), along with a significant decrease in the level of ALP (Alkaline Phosphatase) in the groups treated with dioxin (both low and high doses), with or without silymarin, compared to the control group, as presented in Table (3).
Table (3): Levels of liver enzymes (AST, ALT, ALP) in female albino rats.

Groups	Control	Low-Dose Dioxin	High-Dose Dioxin	Low-Dose Dioxin with Silymarin	High-Dose Dioxin with Silymarin
AST (µ/L)	28.29±0.92	32.556±1.199	32.56±1.199	31.44±1.03	31.95±0.40
ALT (µ/L)	25.05±4.45	28.7±2.1	30.37±0.59	27.25±0.64	29.3±0.78
ALP (µ/L)	114.04±1.19	101.25±4.17	91.49±4.60	104.52±6.29	102.87±1.82

Histological Study
Liver – Control Group: The current study revealed that the liver tissue in the control group appeared histologically normal. The central vein was wide and clearly defined, lined with endothelial cells resting on a basement membrane. Surrounding the central vein, hepatocytes were arranged in radial cords. Each hepatocyte appeared polygonal with a spherical nucleus. Between the rows of hepatocytes, blood sinusoids were visible, containing Kupffer cells (Figure 1).

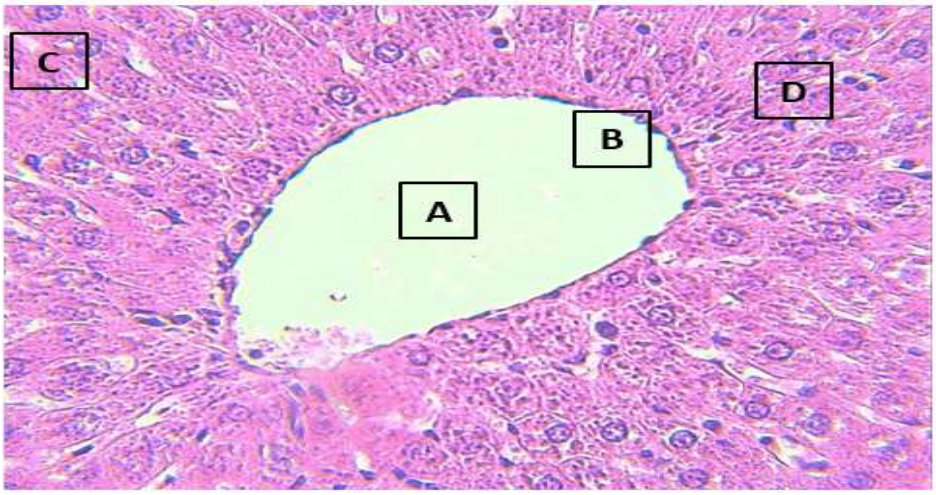

Figure (1): A liver section from the control group showing a wide-lumen central vein (A) lined with the basement membrane and endothelial cells (B), rows of polygonal hepatocytes with spherical nuclei (C), and blood sinusoids containing Kupffer cells (D). Stained with H&E, magnification 40x.

Liver of the group treated with a low dose of dioxin: The current study showed that the liver section exhibited dilation of the blood sinusoids filled with red blood cells. The sinusoids were continuous with the central vein at its periphery and contained abundant Kupffer cells within their lumens. The walls of the central vein were surrounded by a focal aggregation of white blood cells. The hepatocytes appeared in long rows with faint spherical nuclei, and some nuclei were absent (Figure 2).

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https://theaspd.com/index.php

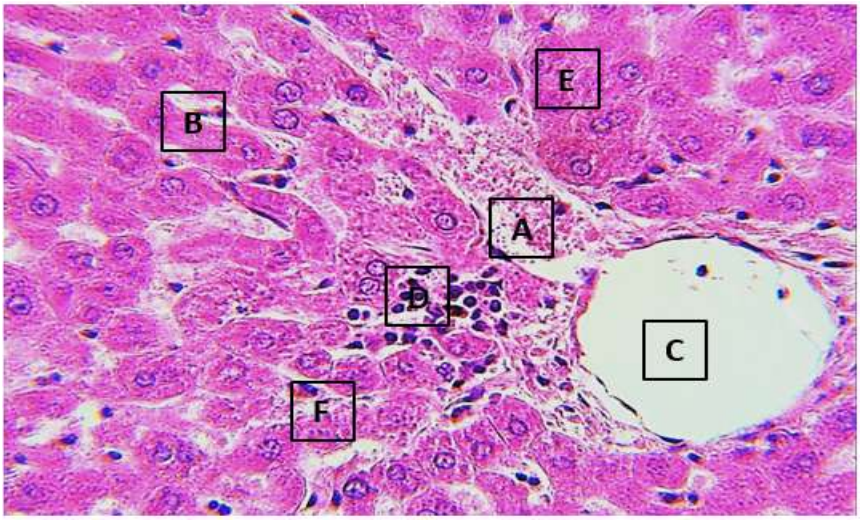

Figure (2): A liver section from the group treated with the standard dose of dioxin showing dilated blood sinusoids filled with red blood cells (A), Kupffer cells (B), the central vein (C) surrounded by a focal aggregation of white blood cells (D), hepatocytes with pale nuclei (E), and degenerated hepatocytes (F). Stained with H&E, magnification X40.

Liver of the group treated with a high dose of dioxin: The current study revealed that the liver tissue showed severe congestion of degraded blood along with large numbers of hemosiderin pigment, some white blood cells, and aggregated inflammatory cells and macrophages around the walls of the central vein. Hepatocytes appeared densely packed, with blood sinusoids in between containing some Kupffer cells (Figure 3).

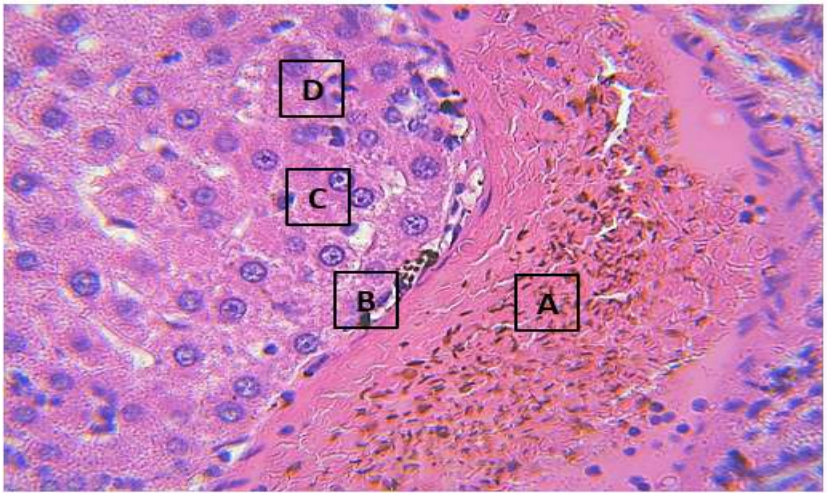

Figure (3): A liver section from the group treated with a high dose of dioxin showing severe blood congestion with hemosiderin pigment and white blood cells (A), infiltration of inflammatory cells and macrophages around the central vein (B), densely packed hepatocytes (C), and Kupffer cells within the blood sinusoids (D). Stained with H&E, magnification 40x.Additionally, the portal area of the liver contained the portal vein with thickened walls, filled with red blood cells, and surrounded by white blood cells and macrophages. Also observed in the portal region was a branch of the bile duct showing epithelial hyperplasia. Hepatocytes appeared in rows surrounded by vascular channels containing Kupffer cells (Figure 4).

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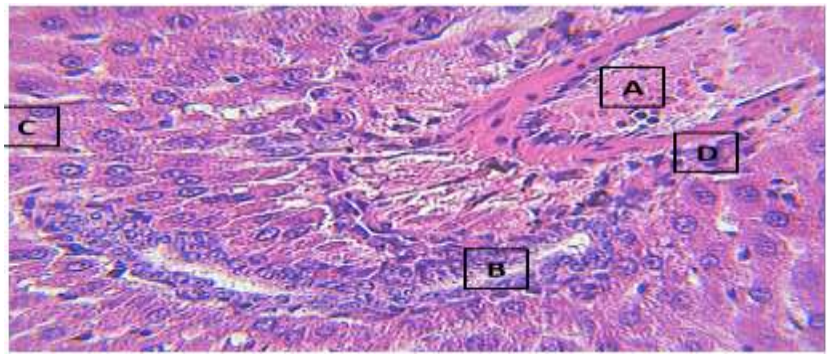

Figure (4): Liver section from the group treated with a high dose of dioxin, showing the portal area with a thickened portal vein wall filled with red and white blood cells (A), a branch of the bile duct exhibiting epithelial cell hyperplasia (B), rows of hepatocytes (C), and white blood cells in the portal area (D). Stained with H&E, magnification 40x.

Liver of the Group Treated with Low Dose of Dioxin Combined with Silymarin: The current study showed that the hepatic lobule contained a central vein with a small thrombus in its lumen and an endothelial lining on the basement membrane. Hepatocytes appeared arranged in rows, each cell polygonal in shape with basophilic-stained spherical nuclei. The sinusoidal network appeared narrow around the hepatocyte membranes and contained Kupffer cells within the sinusoidal spaces (Fig. 5).

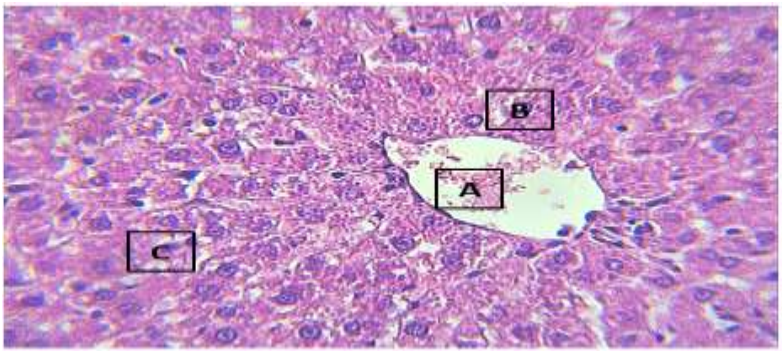

Figure (5): Liver section from the group treated with a standard dose of dioxin and silymarin extract. The hepatic lobule shows a central vein containing a thrombus (A), rows of hepatocytes with darkly stained spherical nuclei (B), and a narrow sinusoidal network containing some Kupffer cells (C). Stained with H&E, magnification 40x.

Liver of the Group Treated with a High Dose of Dioxin Combined with Silymarin: The current study showed that the hepatic lobule contained a dilated central vein lined with endothelial cells resting on a thickened basement membrane, with detachment observed at some edges. Hepatocytes contained foamy cytoplasm surrounding pale spherical nuclei. Numerous white blood cells and macrophages were present in the fibrous connective tissue around the vein. Some dilated sinusoidal spaces containing Kupffer cells were also observed (Figure 6).

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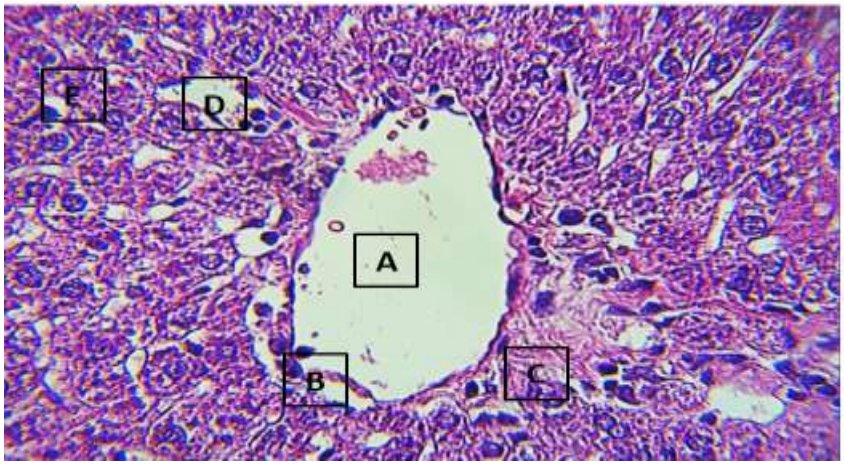

Figure (6): Liver section from the group treated with a high dose of dioxin and silymarin extract. The hepatic lobule shows a dilated central vein lined with simple squamous cells (A), thickened basement membrane (B), infiltration of white blood cells around the central vein (C), dilated sinusoids containing Kupffer cells (D), and hepatocytes with vacuolated cytoplasm (E). Stained with H&E, magnification 40x.

Fetal Images

Control Group Fetuses : No malformations were observed in the fetuses of the control group. Three healthy female albino rats were mated with a single male that had not been exposed to any substances. The results showed healthy, non-deformed fetuses. Dissections were performed 21 days after the detection of the vaginal plug, which corresponds to the expected time near delivery. The number of fetuses was balanced between the uterine horns, with 8–10 fetuses per female, as seen in the images. A fetus from the control group is shown with the following features: Facial area (1), including the optic vesicle (2), Nasal and frontal head regions (3), Smooth parietal head surface (4), Prominent auricular discs (5), Straight back area (6), Anterior limbs extended diagonally and straight (7), Tail slightly curved and tapering at the end (8), Umbilical region showing the connection of the umbilical

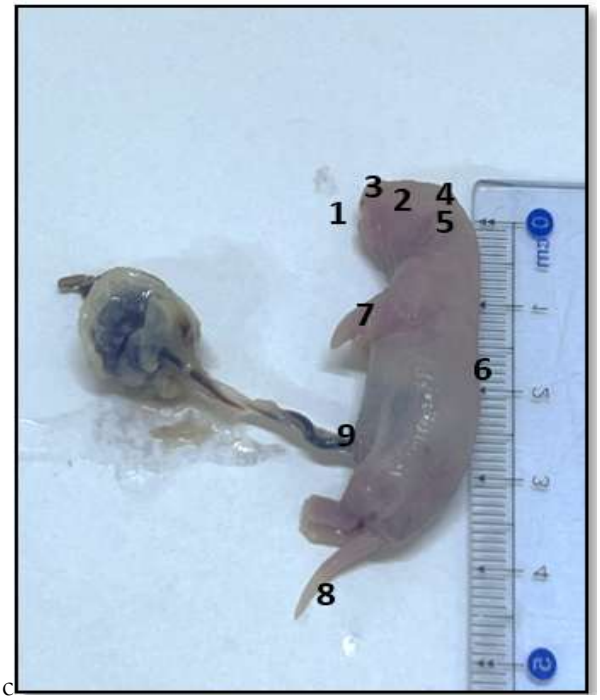

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Image (1): Represents fetuses from the control group.

Image (2): Represents fetuses from the control group.

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ISSN: 2229-7359
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Fetuses of the Group Treated with a Low Dose of Dioxin: Images (3) and (4) illustrate the effects of dioxin at a concentration of 4 µg/kg on the fetuses. In the first female, there were six fetuses, four of which showed malformations compared to the control group. The observed malformations included: Shortened forelimbs (1), Protruding upper jaw area (2), Altered curvature of the spine (3), Smaller overall fetal size (4), Abnormal shape and size of the placenta (5). In the second female, only four fetuses were present, and they showed similar malformations including: Shortened forelimbs (6), Abdominal distension (7). The fetuses from the third female were also smaller in size, with: (8). Fused and shortened forelimbs with missing eye sockets (Image 4), (9). Pyramid-shaped curvature at the top of the head, (10). Tail curved toward the umbilical cord.

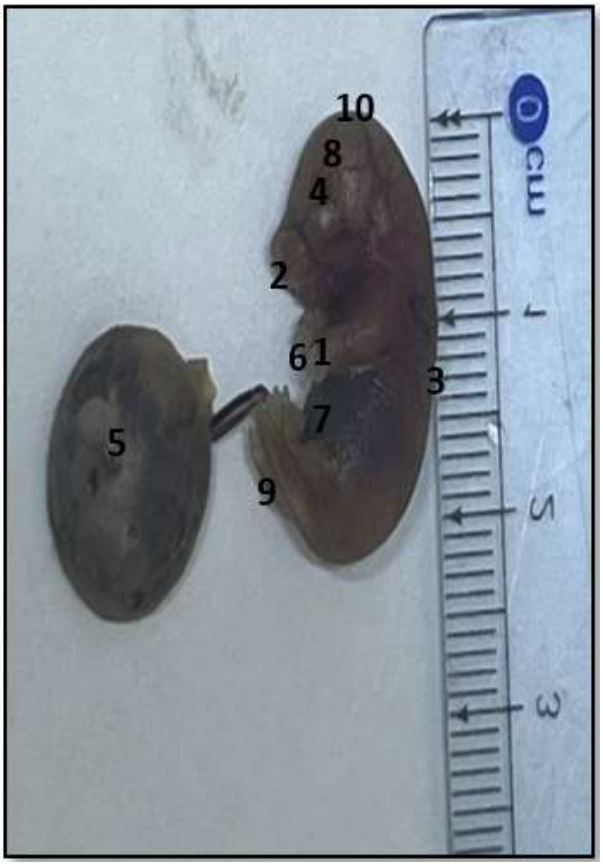

Image (3): Represents fetuses from the second group.

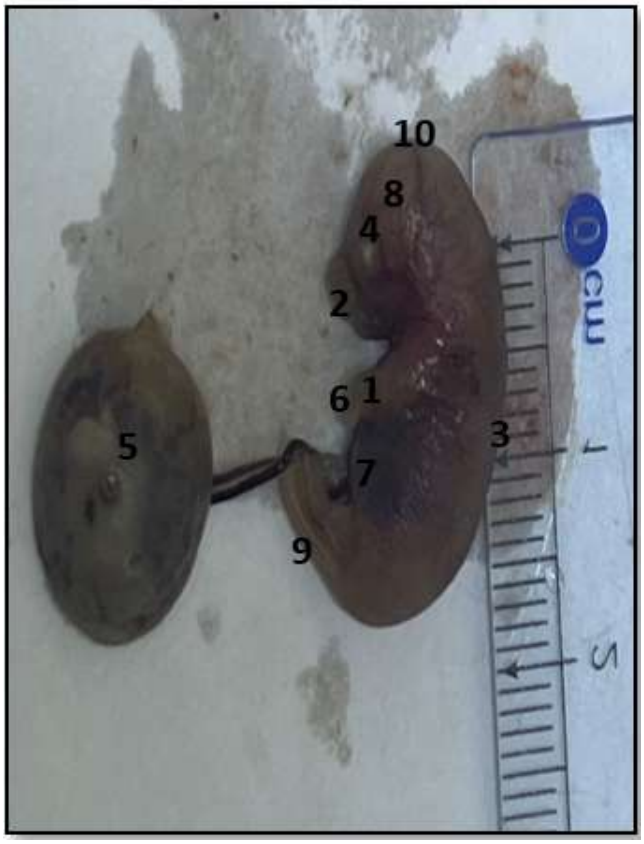

Image (4): Represents fetuses from the second group.

Fetuses of the Group Treated with a High Dose of Dioxin: The effects of dioxin at a concentration of 40 µg/kg on fetuses are clearly observed. Three female albino rats were mated: In the first female, after 20 days of gestation, there were eight fetuses, all of which were dead. In the second female, after 21 days of gestation, only three fetuses were observed, all of which were abnormal. The malformations included: Underdeveloped upper jaw area (1), Shortened forelimbs (2), Abnormally shaped and enlarged placenta with a black coloration and slight swelling (3). In the third female, four fetuses were found. They were also large in size, with noticeable tail curvature and shortening (4).

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Image (5): Represents fetuses from the third group.

Image (6): Represents fetuses from the third group.

Fetuses of the Group Treated with a Low Dose of Dioxin and Silymarin: In Image 7, the fetus shows: The dorsal surface of the head (1), A slight bulge in the neck area (2), Small auditory discs (3), Scarring and multiple areas of congestion on the forelimbs (4), Shortened hindlimbs with absence of digits (5), A longitudinal fold in the abdominal area connected to the umbilical cord (6).In Image 8, the fetus shows: The parietal region of the head (1), A straight slope at the occipital region on the back of the head (2), Small auditory discs with a white scar, which is also visible on the optic vesicle (3), Red scarring on the forelimbs and thickening of the left forelimb, which lacks digits (4), Short hindlimbs also missing digits (5), A skin fold in the umbilical region connected to the umbilical cord (6).

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Image (7): Represents fetuses from the fourth group.

Image (8): Represents fetuses from the fourth group.

Fetuses of the Group Treated with a High Dose of Dioxin and Silymarin: In Image 9, the fetuses show: Mild swelling in the nasal area (1), Shortened jaw region and an elevated forehead surface (2), Slight curvature in the occipital (back) region of the head (3), A straight appearance of the back and lumbar area (4), Optic vesicle atrophy with a faint scar (5), Short and thickened forelimbs with small, atrophied digits (6), A tumorous sac-like structure over the short hindlimbs (7), An upward-curved tail (8), A darkly pigmented area on the abdomen (9). In Image 10, the fetuses show: Hypertrophy (excessive enlargement) of the nasal region (1), A nearly acute, pyramid-shaped curvature of the head's summit (2), Swelling of the optic vesicle (3), Rudimentary auditory discs (4), Forelimbs directed upward from the shoulder joint (5), Wrinkled skin in the abdominal region (6), Hindlimbs curved inward toward the abdomen (7), A long, tapered, upward-curved tail (8).

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Image (9): Represents fetuses from the fifth group.

Image (10): Represents fetuses from the fifth group.

DISCUSSION

The current study demonstrated that groups treated with dioxin, at both low and high doses, exhibited a significant increase in the levels of 8-isoprostane, malondialdehyde (MDA), and 8-hydroxy-2'-deoxyguanosine (8-OHdG), along with a significant decrease in antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These results are consistent with a study by Setianingrum and Hasketto (2021), which showed that tetrachlorodibenzoate-para-dioxin (TCDD), a member of the dioxin family, significantly reduced SOD levels in mice compared to the control group. Similarly, Bryson and Rosen (2024) reported that TCDD exposure led to significant decreases in glutathione, glutathione peroxidase, catalase, SOD, and total antioxidant capacity in the treated groups compared to the control group.These effects may be attributed to the toxic nature of dioxins, persistent organic pollutants that have been banned for decades but are still produced in some countries (Sarkar et al., 2021). Exposure to dioxin (TCDD) increases serum levels of MDA and γ-glutamyl transferase (GGT), two early indicators of oxidative stress, while reducing the activity of key antioxidant enzymes such as SOD, CAT, GSH, and GSH-Px (Setianingrum et al., 2019). Regarding the role of silymarin (SM) in alleviating oxidative stress and increasing antioxidant levels, our results are consistent with those of Celebes and Gedikli (2023). In their study, which used 50, 100, and 200 mg/kg of silymarin to treat sepsis resulting from cecal ligation and puncture, significantly lower levels of MDA and higher levels of GSH-Px, CAT, and SOD were observed in the treated groups compared to the infected group. This protective effect is

attributed to the powerful antioxidant properties of silymarin, a uniform mixture of flavonolignins (70–80%), primarily comprising silibinin, silydianin, and silychristin, with silybinin as the main active component. Silymarin acts as a free radical scavenger, has anti-inflammatory and immunomodulatory effects, and provides protection against UV-induced skin damage (Akhtar et al., 2023). Our results also indicated that dioxin exposure increases liver enzyme levels, consistent with other studies that have shown that dioxin exposure increases levels of AST, ALT, and GGT, key indicators of liver damage and dysfunction. These changes are often accompanied by elevated protein and bilirubin levels and the development of liver fibrosis, suggesting a direct relationship between dioxin exposure and liver damage in people living in contaminated areas (Dobrzyński et al., 2021; Pham et al., 2022). Pham et al. (2022) performed histological analysis of liver samples from 33 patients with chronic hepatitis and found that elevated serum TCDD levels were associated with elevated liver function markers (AST, ALT, total protein, and bilirubin) and advanced stages of fibrosis. Increased TCDD levels were also associated with more severe liver damage, suggesting a potential risk of liver cancer. Gender differences also influence the effects of dioxins. Female mice showed greater increases in liver fat and cholesterol, alterations in gene expression, and histological changes such as hepatic steatosis, inflammatory infiltration, and hepatocyte degeneration, all of which contribute to an increased risk of liver fibrosis and fatty liver disease (Dobrzyński et al., 2021; Bolatimi et al., 2024).Regarding the role of silymarin in reducing liver enzyme levels compared to dioxin-treated groups, our results are consistent with those of Abdel-Maboud et al. (2021), who showed that silymarin significantly reduced levels of AST, ALT, and alkaline phosphatase. This was accompanied by changes in the expression of alpha-smooth muscle actin in fibrotic tissue, suggesting that early administration of recommended doses of silymarin can inhibit fibrosis mechanisms and slow its progression. Doses of 50 mg and 200 mg of silymarin were effective in this regard (Gillesen and Schmidt, 2020). Our histological results also confirmed the changes in liver tissues of dioxin-treated female mice, consistent with the findings of Dobrzynski et al. (2021), who reported cytoplasmic vacuoles in hepatocytes, unclear cell boundaries, hepatocyte disintegration, foamy cytoplasm, and hyperchromatinized nuclei in TCDD-treated female neonates. Additionally, various infiltrating cells were observed in the central vein and hepatic triad. Dioxins stimulate the expression of detoxifying enzymes such as flavin-containing monooxygenase 3 (FMO3), which regulates the liver's oxidative stress response and lipid metabolism. Genetic factors like the presence or absence of FMO3 influence the liver's response to dioxin-like pollutants, impacting oxidative stress and lipid storage (Agarwal *et al.*, 2024). Dioxins disrupt cholesterol and fat metabolism, leading to impaired cholesterol synthesis, triglyceride accumulation, and fatty acid metabolism dysfunction (Liang *et al.*, 2021; Kumbale *et al.*, 2023).Silymarin, a lipophilic active compound, contains three flavonolignan isomers—silibinin, silychristin, and silydianin—and is considered a physiologically active component (Sapthasri, 2021).Regarding silymarin's role in improving histological changes in the liver, its beneficial effects on liver cells have been observed in patients with conditions such as nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), and cirrhosis. Silymarin exerts its hepatoprotective effects by modulating oxidative stress, reducing liver fat accumulation, and lowering blood insulin levels. It also improves liver function and reduces liver toxicity caused by excessive acetaminophen intake. Furthermore, animal studies, such as those conducted by Adelina (2022), have demonstrated silymarin's ability to reduce oxidative stress in laboratory mice. In humans, exposure to various chemicals can lead to increased oxidative stress, which may contribute to drug-induced liver damage. Under normal conditions, free radical production and the body's ability to activate antioxidant enzymes work together as an integrated defense system. The antioxidant properties of silymarin provide an effective preventive strategy against chronic liver diseases, such as jaundice, cirrhosis, and hepatitis, by preventing liver dysfunction and promoting the restoration

of normal liver function. This protective effect has been demonstrated in studies on carbon tetrachloride-induced liver toxicity in rats, where silymarin reduced oxidative stress and prevented glutathione deficiency (Villanueva-Paz et al., 2021).Our results also showed congenital malformations in rat fetuses due to maternal exposure to dioxin. Dioxin exposure can lead to various adverse effects, including gastrointestinal, liver, and breast cancers, growth disturbances, hepatic injury, and congenital anomalies such as cleft palate, kidney malformations, immunotoxicity, neurotoxicity, cardiac diseases, vomiting, respiratory problems, reproductive disorders, hypertension, and asthma-like symptoms. Dioxins can also cause DNA mutations, generate free radicals, and promote lipid peroxidation (Abdulkareem and Nanakali, 2020). Dioxins and dioxin-like compounds are highly toxic persistent organic pollutants (POPs) that resist chemical, biological, and photodegradation (Prabhu and Lakshmipraba, 2022). Foods, particularly animal-based products, are commonly contaminated with dioxins. Dioxin toxicity levels are measured as international toxic equivalents (TEQs) (Esser *et al.*, 2021) and accumulate in the food chain through absorption and storage in adipose tissues (González *et al.*, 2021).Exposure to dioxins during pregnancy is associated with many harmful effects on fetal development. Key findings indicate that dioxins may impair fetal growth, disrupt hormonal balance, alter neurodevelopment, and affect placental function and the immune system, leading to poor fetal growth, developmental delay, and increased risk of low birth weight (Long *et al.*, 2022; Mahfouz *et al.*, 2024). In Animal studies have shown that prenatal exposure to dioxins suppresses fetal growth hormone production, leading to lower body weight, shorter gestation, and developmental problems after birth. These effects are associated with alterations in pituitary cell proliferation in the fetus, achieved through activation of the aryl hydrocarbon receptor (AhR) pathway (Hattori et al., 2021; Iqbal et al., 2021).Silymarin's role in reducing fetal malformations is due to its antioxidant and anti-inflammatory properties, which provide protective effects for several organs, including the reproductive system. However, direct evidence regarding its effects specifically on fetuses is limited. Most available studies focus on its safety profile, its protective effects against reproductive toxicity, and its effect during pregnancy and lactation in animal models. Silymarin is generally considered non-toxic, even at relatively high doses, and is safe for use in various therapeutic contexts, including reproductive health. Regarding reproductive toxicity, silymarin shows protective effects against damage caused by chemotherapy drugs and heavy metals, due to its antioxidant, anti-inflammatory, and anti-apoptotic properties. These mechanisms help reduce oxidative damage and inflammation in reproductive organs, which may indirectly benefit fetal development by preserving reproductive system health (Hariyanti *et al.*, 2023; Kordedeh *et al.*, 2024).Supplementing with silymarin during late pregnancy and lactation in studies on pregnant and lactating sows led to reduced inflammatory responses and altered gut microbiota composition, which were associated with improved maternal health and potentially positive outcomes for offspring, although direct effects on fetuses were not measured (Xu *et al.*, 2022). No evidence from reviewed studies suggests that silymarin causes embryotoxicity. Its long history of use and the absence of reported toxicity even after long-term intake support its favorable safety profile (Koltai and Fliegel, 2022; Hariyanti *et al.*, 2023). Key mechanisms of silymarin include reducing oxidative stress and inflammation, stabilizing cell membranes, and modulating immune responses, which are believed to underlie its protective actions in reproductive and other tissues (Wadhwa *et al.*, 2022; Xu *et al.*, 2022).Due to its excellent therapeutic efficacy, silymarin is among the most widely used dietary supplements, with approximately 75 commercial brands available in various dosage forms (tablets, capsules, syrup, etc.). Nano-silymarin has been approved by the Vietnamese Food and Drug Administration using the ionic gelation technique. Researchers reported that this developed formulation showed significant antifibrotic activity against carbon tetrachloride-induced liver damage (Abdullah *et al.*, 2022).

CONCLUSION:

This study provides compelling evidence that exposure to dioxin induces significant oxidative stress, hepatotoxicity, and developmental abnormalities in rat fetuses. The observed biochemical alterations—such as elevated levels of MDA, 8-isoprostane, and 8-OHdG, along with reduced antioxidant enzyme activities (SOD, CAT, and GPx)—corroborate the histopathological findings of hepatic degeneration and fetal malformations. These results underscore the hazardous impact of persistent organic pollutants like dioxins on both maternal and fetal health. Conversely, silymarin co-administration markedly mitigated these toxic effects, as demonstrated by improved antioxidant defense, reduced hepatic enzyme levels, preserved liver histoarchitecture, and a lower incidence of fetal deformities. The protective effects of silymarin are primarily attributed to its potent antioxidant, anti-inflammatory, and cytoprotective properties. In conclusion, silymarin shows promising potential as a therapeutic agent in preventing dioxin-induced oxidative damage and associated hepatic and developmental toxicity. Further studies, especially clinical trials, are warranted to explore its efficacy in humans and to establish standardized dosages and treatment protocols for populations at risk of dioxin exposure.

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