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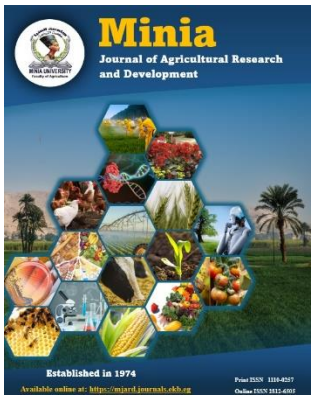

Silymarin and Nano Silymarin alleviate the oxidative stress and damaged Histoarchitecture of the liver in Ehrlich Ascites carcinoma bearing mice

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ABSTRACT

The ancient medicinal plant *Silybum marianum* has been used to cure various diseases for centuries. Silymarin is the main bioactive ingredient found in these plant seeds. Thus, the purpose of this study was to evaluate the ability of silymarin and nano silymarin to alleviate oxidative stress and ameliorate damaged histoarchitecture of the liver in Ehrlich ascites carcinoma-bearing mice. HPLC analysis of nano silymarin flavonoid compounds in milk thistle seed extract was performed. A total of 60 Swiss albino female mice were randomly separated into four equal groups. The first group was assigned as the normal control. An intraperitoneal injection of 2.5 x 10^6 EAC cells was administered to each of the three remaining groups. As the EAC positive control group, the second group didn't receive additional treatment. Mice in the third and fourth groups ingested orally Silymarine and nano silymarine at a dose of 100 mg/kg b.w. daily for 6 weeks. Markers of oxidative stress and tumors, as well as enzymatic and non-enzymatic antioxidants, were assessed in each experimental group. Furthermore, a liver histopathological examination was performed. The findings showed that mice with induced EAC had damaged liver histoarchitecture, low levels of enzymatic and non-enzymatic antioxidants, and high levels of tumor and oxidative stress markers. Administration of silymarine and nano silymarine to EAC-bearing mice reduced oxidative stress and improved the histoarchitecture of the damaged liver.

Keywords: EAC-bearing mice; Nano silymarin; oxidative stress; Silymarin.

1. INTRODUCTION

The utilization of natural materials for medicinal purposes has attracted much attention throughout the last two decades. Liver diseases have been effectively treated with *Silybum marianum*, a natural medicinal plant for decades (Gioti et al., 2019; Takke & Shende, 2019). Originally from the highlands of the Mediterranean, Asia, and North Africa, *Silybum marianum* is now grown all over the world (Hemieda et al., 2016; Park et al., 2015; Sheweita, Abd El-Gabar, & Bastawy, 2001). The most prevalent type of silymarin is a concentrated

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extract, while it is often said to be derived from the seeds of the well-known Milk Thistle plant (Lto, Tamano, & Shirai, 2003). Silymarin, the therapeutic component of *Silybum marianum*, is a complex mixture of flavonoids, flavonolignans, and polyphenolic compounds (Javed, Kohli, & Ali, 2011). These substances have several other biological properties in addition to acting as antioxidants (Surai, 2015). The most prevalent and biologically active of the four main flavonolignan isomers identified in Silybum marianum are silibinin, isosilibinin, silichristin, and silidianin (Abenavoli et al., 2010; Saller, Meier, & Brignoli, 2001). The antioxidant activities of silymarin and silibinin are known to reduce pro-inflammatory cytokines in addition to their hepatoprotective benefits (Federico, Dallio, & Loguercio, 2017). The *in vitro* and *in vivo* cancer models, including those of the prostate, bladder, liver, breast, lung, and kidney, were applied to investigate the anticancer properties of silymarin and silibinin (Parashar et al., 2019; Yassin et al., 2021). Numerous techniques, including DNA repair, cell cycle arrest, growth and proliferation suppression, antiangiogenic impacts, and invasion and metastasis prevention, have been used during silymarin and silibinin therapy (Hosseinabadi et al., 2019; Zappavigna et al., 2019).

Silymarin is widely recognized for its anti-inflammatory and antioxidant abilities, but it also exhibits several beneficial biological and pharmaceutical properties, including immunological and neurotrophic regulation, protein synthesis promotion, cardiovascular, and neurological protection. Additionally, silymarin exhibits anticancer properties in human adenocarcinoma cell lines, including in the areas of liver metabolism and cell regeneration in cases of toxic liver damage. Silymarin demonstrates anti-diabetic properties and has hypolipidemic and antifibrotic advantages in chronic inflammatory liver disease (Bijak, 2017; Javed, Kohli, & Ali, 2011). It has been used historically for more than two millennia to treat conditions affecting the kidneys, spleen, gall bladder, and mainly the liver (Abenavoli & Milic, 2017). As a dietary supplement, it is widely used nowadays to help treat liver disorders (Abenavoli et al., 2018; Tajmohammadi, Razavi, & Hosseinzadeh, 2018). A recent study summarizes the numerous investigations conducted to ascertain the biological and pharmacological characteristics of silymarin in liver treatment, as well as its antioxidant and anti-inflammatory activities (Gillessen & Schmidt, 2020).

Even though silymarin has hepatoprotective properties, its greatest drawback when taken orally is its low absorption (Woo et al., 2007). The limited accessibility of silymarin through the gut's epithelial cells, its quick excretion, and its inability to dissolve in water in stomach acidity could all contribute to its inadequate bioavailability (Clichici et al., 2020). Several silymarin preparations have been created to boost the compound's bioavailability and get around this problem. The application of nanotechnology could significantly enhance the bioavailability of silymarin and its medicinal benefits (Ma et al., 2017)Hence, the goal of the present investigation was to evaluate the biological impacts of silymarin and nano silymarin on improving the oxidative stress and damaged histoarchitecture of the liver in Ehrlich ascites carcinoma-bearing mice.

2. MATERIALS AND METHODS

2.1. Extract preparation

The seeds of *S. marianum* were purchased from the Natural Herbs Market in Cairo (Harraz), Egypt. They were then

sliced, dried in the shade, and extracted using ethanol 95%. Reduced pressure was used to extract the dry extract (2.1% yield w/w), which was subsequently blended with water to prepare an aqueous suspension.

2.2. Sillymarin nanoparticles formation

The PQ-N2 Planetary Ball Mill, Gear Drive 4, 220 v, was used to form amorphous silymarin powders in nanoparticle size utilizing the ball milling mechanical approach. 250 stainless steel balls of various diameters are used in equal quantities for grinding at the National Research Center. Using a high-energy ball mill with a chamber environment, the grinding operation was carried out for 90 minutes at a spin speed of 40,000 rpm while the ball's weight was reduced to powder (10: 1). The products are filtered to get rid of contaminants and separate the balls. 100 gm of milled nanopowder was utilized to improve processability or solubility, particularly for those with poor aqueous solubility, (Loh, Samanta, & Heng, 2015). In addition to mechano-chemical changes, the particles' size, specific surface area, and shape might also be altered. As stated before, the supernatant was concentrated after the nano silymarin extract was filtered by the Whatman paper.

2.3. HPLC analysis

The HPLC analysis of nano silymarin flavonoid compounds in milk thistle seed extract was carried out using an Agilent 1260 series. Zorbax Eclipse Plus C18 column (4.6 mm x 250 mm i.d., 5 μm) was used for the separation process. The mobile phase included the following: 0.5:35:65 phosphoric acid : methanol : water (A), and (0.5:70:30) phosphoric acid, methanol: water (B) for 37 minutes at a flow rate of 1 ml/min. At 288 nm, the diode array detector was set. Each sample solution had an injection volume of 10 μl. The temperature of the column was kept at 40°C.

2.4. Tumor cells and experimental animals

The National Cancer Institute's animal house at Cairo University provided Ehrlich Ascites Carcinoma (EAC) cells, which were found to be hyperinflated in female mice (Vincent & Nicholls, 1967). The mice were housed in 38 cm by 23 cm by 10 cm polyacrylic cages. Mice were housed in a standard environment with a 12-hour light/dark cycle. They were provided with a daily supper and free access to water. The mice were acclimated to the laboratory setting for seven days before the start of the trial.

2.5.Transplanting tumor into experimental animals

Briefly, 2.5 × 106 cells/mice were transplanted weekly intraperitoneally to maintain EAC cells. Then cells were harvested from 18–20 g Swiss albino donor mice and suspended in sterile isotonic saline. A predetermined quantity of viable cells (usually 2 × 106 cells/ 20 g body weight) was injected intraperitoneally into each recipient mouse (Gothoskar & Ranadive, 1971). The vitality of the cells was found to be 99% in the Trypan blue exclusion experiment. Seven to eight days following the onset of the tumor-bearing cells, the ascitic fluid was extracted from mice with Ehrlich ascites carcinoma tumors. The animals were administrated intraperitoneally with 0.2 ml of a tumor cell suspension that contained 2.5 × 106 tumor cells. EAC cells originated from volunteer female Swiss albino mice weighing 18–20 g and suspended in sterile saline. A specific quantity of viable cells typically 2.5 × 106 cells/mice were placed in the peritoneal cavity of each recipient mice (Salem, Badr, & Neamat-Allah, 2011). Each recipient mice

received 0.5 ml of EAC cells, which was extracted using a sterile disposable syringe, diluted with 4.5 ml of normal saline, and then given intraperitoneally. The growth of the tumor cells within the peritoneal cavity was allowed.

2.6. Experimental treatments

Sixty Swiss albino female mice, each weighing between 20 and 22 g, were divided randomly into four groups (15 mice each). The first group was assigned as a negative control group and housed in cages and fed a typical meal without any special care. The second group was designated as the tumor-bearing group and maintained in cages on a regular, conventional diet and received intraperitoneally 0.2 ml of ascetic fluid containing $2.5x10^6$ EAC cells. The third and fourth groups were administered by oral Silymarine and nano Silymarine at a dose of 100 mg/kg b.w. every day for six weeks, respectively after receiving 0.2 ml of ascetic fluid containing $2.5x10^6$ EAC. At the end of the experimental trial, the mice were anesthetized and their axillary plexus was punctured to collect animal blood. Serum samples were obtained to use the Cobas 6000 analyzer series for determining and analyzing various blood biochemical parameters.

2.7. Biochemical assessments

Alpha-fetoprotein (AFP) tumor marker, Lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PD), Superoxide dismutase (SOD), Catalase (CAT), Glutathione (GSH), and malondialdehyde (MDA) were measured using an automated diagnostic Cobas 6000 analyzer. The Cobas 6000 analyzer series is a powerful tool for complete diagnostic laboratory automation. It is a fully automated, software-controlled, random-access instrument for photometric and immunoassay analysis that is intended for qualitative and quantitative in vitro assessments through a range of assays.

2.8. Histopathological examination

The livers of the treatment and control groups were cleaned and dehydrated with increasing alcohol grades after being fixed and sliced in a 10% natural formalin solution. The specimens were cleaned in xylene, embedded in paraffin, sectioned at a thickness of 3-6 microns, and stained with hematoxylin and eosin (H&E) before being studied under a microscope for histological investigations (Banchroft, Stevens, & Turner, 1996).

2.9. Statistical analysis

The statistical analysis was conducted using the GraphPad Prism 8 program and included Tukey's multiple comparison tests (Tukey, 1951) and one-way analysis of variance (ANOVA), with a 95% confidence interval of $P < 0.05$. The mean SEM is used to display the results. It was determined that there was a statistically significant difference between the treatment and control groups.

2.10. Ethical Statement

All procedures were performed by the ethics committee for the care and use of animals, microorganisms, and living cell cultures in education and scientific research at the Faculty of Agriculture, Minia University, El-Minya 61519, Egypt (Approval No: MU/FA/010/12/22).

3. RESULTS AND DISCUSSION

3.1. Identification of nano silymarin flavonoid compounds in milk thistle seed extract using HPLC

HPLC, or high-performance liquid chromatography, is the most used technology for determining the chemical

compositional differences between medicinal herbal samples (Wang et al., 2016). Multiple HPLC techniques have been recorded to examine milk thistle (*Silybum marianum*) bioactive flavonoid compounds. The literature reported on the HPLC analysis of the four flavonolignans (Silybin B, Iso silybin B, Silychristin, and Silydianin) in silymarin (Ding et al., 2001; Kvasnička et al., 2003; Mesbah, Khalifa, & Tawfik). Silybin diastereoisomers A and B have been investigated by a UV detector and HPLC (Rickling et al., 1995). Silymarin is one of the most investigated plant extracts with confirmed mechanisms of action worldwide. The chemistry of milk thistle is well known, and the pharmacological effects are probably triggered by silymarin and its components, mainly silybin, a free radical scavenger, and antioxidant (El-Shafei et al., 2023; Shaker, Mahmoud, & Mnaa, 2010).Thus, the present study examined the nano silymarin flavonoid compounds in milk thistle seed extract. High concentrations of Silybin B (332.995 µg/g extract), Silychristin (210.682 µg/g extract), and Silybin A (207.135 µg/g extract) are displayed in the nano silymarin mixture as indicated in **Figure 1** and **Table 1**. This may help to confirm the reason nano silymarin is more active than silymarin, as will be apparent in the subsequent findings. The mixture also contained lower ratios than Silydianin (111.394 µg/g extract), Iso Silybin A (92.625 µg/g extract), and Iso Silybin B (45.168 µg/g extract).

Table 1: HPLC analysis of nano silymarin flavonoid compounds in milk thistle seed extract

Identified compounds	Retention time (min)	Conc. (µg/g extract)
Silychristin	16.13	210.682
Silydianin	18.20	111.394
Silybin A	24.91	207.135
Silybin B	26.09	332.995
Iso silybin A	28.48	92.625
Iso silybin B	29.18	45.168
Taxifolin	ND*	ND*

*ND not detected

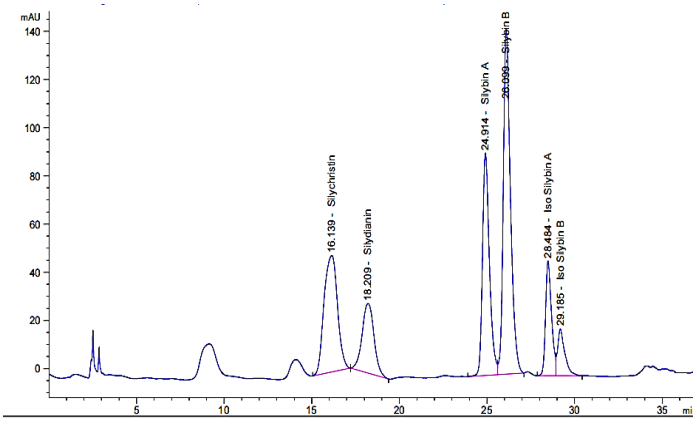

Figure 1: HPLC chromatogram of nano silymarin flavonoid compounds in milk thistle seed extract

3.2. Tumor and oxidative stress markers

Tumor markers are biomarkers that can be raised in bodily tissues, blood, or urine when one or more cancer types are present. Numerous tumor markers are employed in oncology to assist in identifying the presence of cancer; each one represents a distinct disease process. Cancer may be indicated by a high level of a tumor marker, although other factors can also cause false positive readings. The existence of a tumor can cause non-tumor cells to generate tumor markers, or the tumor itself may produce them (Nagpal et al., 2016). The elevated levels of Alpha-Fetoprotein (AFP) in EAC mice (Group 2), as shown in **Figure 2**, could support a diagnosis of cancer of the liver, ovaries, or testicles. The difference between the EAC mice and the control was very highly significant ($P < 0.001$). Nano silymarin demonstrated a clear recovery compared to EAC values, reaching 43.72% in the highly significant record ($P < 0.01$), whereas silymarin use resulted in a significant difference of 41.85% ($P < 0.05$) **(Figure 2)**.

The primary regulating enzyme in the hexose monophosphate shunt, glucose-6-phosphate dehydrogenase (G6PD), stimulates the conversion of glucose-6-phosphate (G6P) to 6-phosphogluconolactone and the generation of reducing substitutes in the form of NADPH to fulfill the demands of reductive biosynthesis and cellular redox status (Scott et al., 1991).

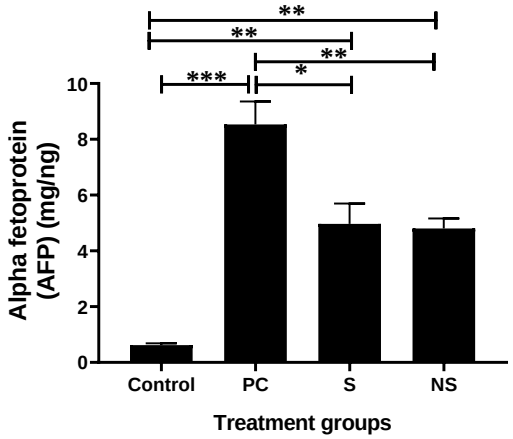

Figure 2: The concentration of serum alpha-fetoprotein (g/l) of Ehrlich ascites carcinoma mice ingested silymarin and nano silymarin orally in different experimental groups. Values are presented as mean ± SEM, * $P < 0.05$ significant differences, ** $P < 0.01$ highly significant differences, *** $P < 0.001$ very high significant differences, **** $P < 0.0001$ extremely high significant differences. PC = positive control, S = Silymarin, NS = Nano silymarin.

Further, G6PD expression determines the difference in sensitivity between males and females to heat-induced oxidative stress. The importance of G6PD in preserving a healthy redox balance and providing resistance to harmful circumstances is underlined (Pérez - Crespo et al., 2005). G6PD and cancer have an unclear relationship. Previous studies found increased G6PD activity in tumors from a variety of malignancies (Bezwoda et al., 1985; Sun, 1990). Therefore, the reduction of G6PD in the current investigation with nano silymarin administration (Group 4) indicates a reduction in oxidative damage **(Figure 3A)**. EAC or the positive control

group (Group 2) demonstrated a highly significant ($P < 0.0001$) negative impact. Comparing the nano silymarin group to the EAC group, clear data showed that there was a 40.55% improvement in G6PD and a 17.92% improvement in silymarin group **(Figure 3A)**.

The enzyme lactate dehydrogenase (LDH) is extensively distributed and plays a role in the metabolism of carbohydrates by catalyzing the interconversion of lactate and pyruvate using the Nicotinamide adenine dinucleotide (NADH) coenzyme system. Low concentrations of LDH are found in the lung, brain, and smooth muscle, whereas high concentrations are found in the heart, liver, skeletal muscle, erythrocytes, and kidney (Untari et al., 2023). Tissue injury is typically indicated by elevated levels of the LDH enzyme. The enzyme LDH transfers from the cytoplasm of cells to the bloodstream as a result of diseases that destroy tissue (Klein et al., 2020). However, LDH has the potential to be very important in regulating the redox state of cells. LDH can exhibit both pro-oxidative and antioxidative properties in cancer cells at the same time (Gao, Patil, & Jia, 2021). In the current experiment, this marker was highly significantly ($P < 0.0001$) elevated in EAC mice (Group 2) compared to the control animals. Approximate improvement has also been observed in silymarin and nano silymarin groups compared to EAC animals; very similar values were found (22.59 and 22.25%, respectively) **(Figure 3B)**.

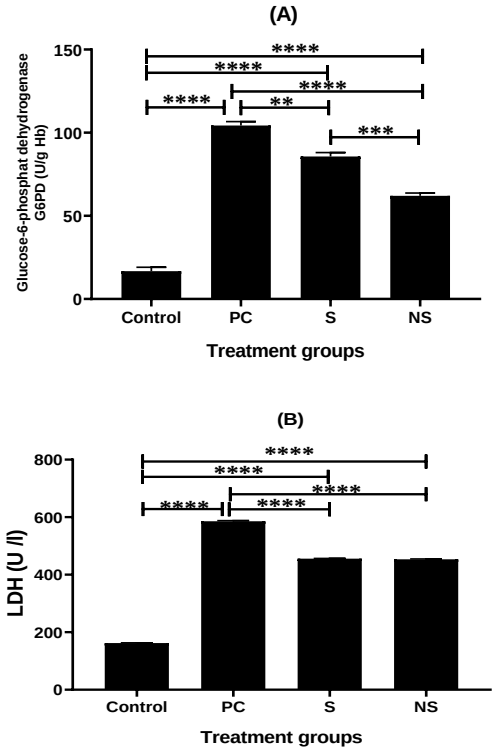

Figure 3: The activities of G6PD (U/g Hb) and LDH (U/l) of Ehrlich ascites carcinoma mice ingested silymarin and nano silymarin orally in different experimental groups. Values are presented as mean ± SEM, * $P < 0.05$ significant differences, ** $P < 0.01$ highly significant differences, *** $P < 0.001$ very high significant differences, **** $P < 0.0001$ extremely high significant differences. PC = positive control, S = Silymarin, NS = Nano silymarin.

The macromolecules most impacted by oxidative stress-induced deficits are lipids, particularly polyunsaturated fatty acids with many carbon-carbon double bonds. Lipid peroxidation produces malondialdehyde (MDA) as an end product of lipid peroxidation, which has been used as a biomarker to assess oxidative stress in a variety of biological samples, including blood, urine, and others, in patients with a variety of diseases, such as cancer, heart disease, lung conditions, and neurological disorders (Del Rio, Stewart, & Pellegrini, 2005; Merendino et al., 2003). In the present study, nano silymarin ingestion reduced the MDA of EAC mice (Group 4), and compared to the control a highly significant ($P < 0.01$) increase has been shown for EAC mice (Group 2). The reduction in MDA value reached approximately MDA in negative control. For nano silymarin and silymarin, ratios were 26.31 and 24.83%, respectively compared to EAC mice **(Figure 4)**.

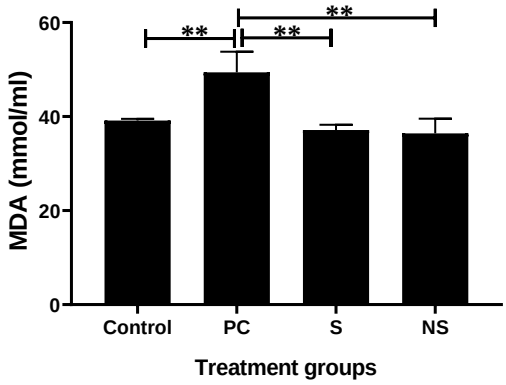

Figure 4: The concentration of serum MDA (µmol/ml) of Ehrlich ascites carcinoma mice ingested silymarin and nano silymarin orally in different experimental groups. Values are presented as mean ± SEM, * *P* < 0.05 significant differences, ***P* < 0.01 highly significant differences, ****P* < 0.001 very high significant differences, *****P* < 0.0001 extremely high significant differences. PC = positive control, S = Silymarin, NS = Nano silymarin.

Our findings are consistent with those of Ramasamy and Agarawal (Ramasamy & Agarwal, 2008) and Kiruthiga et al. (Kiruthiga et al., 2007). By enhancing the activities of antioxidant enzymes, they demonstrated the ability of plant components to scavenge and capture biological reactive oxygen species (ROS) or oxidants and lower the amount of malondialdehyde (MDA).

3.3. Enzymatic and non-enzymatic antioxidants

The most crucial defensive mechanism against oxidative stress-induced cell damage is provided by antioxidant enzymes, which are proteins that catalytically convert reactive oxygen species and their byproducts into stable, harmless compounds (Sáez & Están-Capell, 2014). Catalase is one of the most important antioxidant enzymes found in all aerobic organisms. In cells under

environmental stress, it has been reported to efficiently catalyze H_2O_2 into water and oxygen. It plays a crucial role in protecting the cell from reactive oxygen species (ROS)-induced oxidative damage (Chelikani, Fita, & Loewen, 2004). Comparing the current findings with EAC tumor-bearing mice for CAT showed one of the most satisfactory results. When comparing EAC mice (Group 2) to control animals, a very high and significant (P < 0.001) decrease was found. The biological redox system revealed a non-significant increase for silymarin (23.9%) and a significant increase for nano silymarin (37.5%) when compared to EAC animals **(Figure 5A)**.

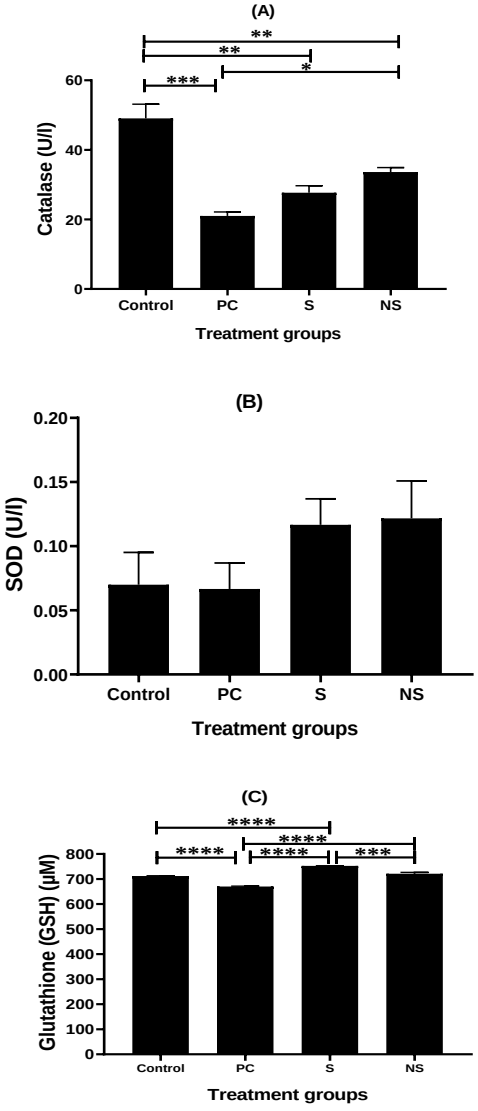

Figure 5: The activities of catalase (U/l), SOD (U/ml), and concentration of serum GSH (nmol/l) of Ehrlich ascites carcinoma mice ingested silymarin and nano silymarin orally in different experimental groups Values are presented as mean ± SEM, * P < 0.05 significant differences, ** P < 0.01 highly significant differences, *** P < 0.001 very high significant differences, **** P < 0.0001 extremely high significant differences. PC = positive control, S = Silymarin, NS = Nano silymarin

Superoxide dismutase (SOD) is another important antioxidant enzyme. It is an enzyme that catalyzes the dismutation of the superoxide (O_2^-) radical into hydrogen peroxide (H_2O_2) and regular molecular oxygen (O_2) alternatively. Other enzymes like catalase break down hydrogen peroxide, which is similarly harmful. SOD is therefore a crucial antioxidant defences in almost all live cells that come into contact with oxygen (Sáez & Están-Capell, 2014). The EAC tumor-bearing group in Figure 4B displayed a slightly reduced SOD activity than the negative control group, which is regarded as a non-significant difference. In contrast, silymarin and, more importantly, nano silymarin increased SOD activity in a non-significant amount as compared to EAC mice. The most significant potential impact is demonstrated by the SOD activity, which was 50% for nano silymarin and 45.4% for silymarin when compared to the EAC group **(Figure 5B)**.

Reduced glutathione (GSH) is one of the most important non-enzymatic antioxidants. It is a tripeptide consisting of a gamma peptide bond that connects the cysteine with the carboxyl group of the glutamate side chain. Glycine is linked to the carboxyl group of the cysteine residue by a common peptide bond. It can prevent vital cellular components from damaging by reactive oxygen species including free radicals, peroxides, and lipid peroxides (Pompella et al., 2003). As shown in **Figure 5C**, nano silymarin was responsible for recovering in high significance ($P < 0.0001$) the GSH value of EAC mice (Group 4) by 7.05%, while serum GSH levels in the EAC tumor-bearing group (Group 2) were significantly ($P < 0.0001$) lower than those in the control group. Silymarin was the only outcome in our work to surpass the nano silymarin value, with an improvement ratio of 10.9% (Group 3).

Our findings demonstrated that nano silymarine had elevated catalase, superoxide dismutase, and reduced glutathione in comparison to the EAC group **(Figure 5A, B, C)**. Nanoparticles are therapeutically effective in Ehrlich's ascites carcinoma EAC mice (Kono et al., 2022). The findings concurred with those of (Ahmadzadeh et al., 2017). They reported that when male rats were given gold nanoparticles, silymarin decreased oxidative stress. Gold nanoparticle-treated animals had higher MDA concentrations and lower CAT and GPX activity. In contrast to the group that received only gold nanoparticles, the group that received silymarin and gold nanoparticles saw an increase in CAT and GPX and a decrease in MDA concentration. They proposed that in diabetic rats, silymarin reduced the toxicity caused by gold nanoparticles. Moreover, according to (Yousefdoost et al., 2019), silymarin nano capsulated against carbon tetrachloride reduced particle size to 169 nm by sonication. In contrast to intestinal fluid, it exhibited greater resistance to gastric fluid. SOD expression was controlled by silymarin and nanocapsules. Thus, the hepatotoxicity caused by oxidative stress has been lessened by capsules.

3.4. Histopathological alterations

Hepatic lobules composed of radiating plates, cords, or strands of cells forming a network surrounding a central vein were visible upon liver examination for control animals **(Figure 6A)**. The liver strands drastically stretch along the liver lobules and alternate with thin blood sinusoids. The centrilobular vein in control often has a circular shape and is bounded by a thin layer of endothelial cells held up by a small

number of collagen fibers. EAC mice liver slices displayed active Kupffer cell cytoplasmic vacuolation of hepatocytes, leucocytic infiltration, and a very congested portal vein **(Figure 6B)**. The liver tissue from EAC mice treated with silymarin had a normal architecture, providing the liver sections with a rather healthy appearance. In this group, the bile duct deteriorated and the portal vein was clogged. The cytoplasm, nuclei, and hepatocytes were largely normal, but the blood sinusoids were dilated **(Figure 6C)**. The total absence of fibrosis in liver sections from EAC treated with nano silymarin demonstrated an advanced level of protection for hepatic tissues against the harmful effect **(Figure 6D)**.

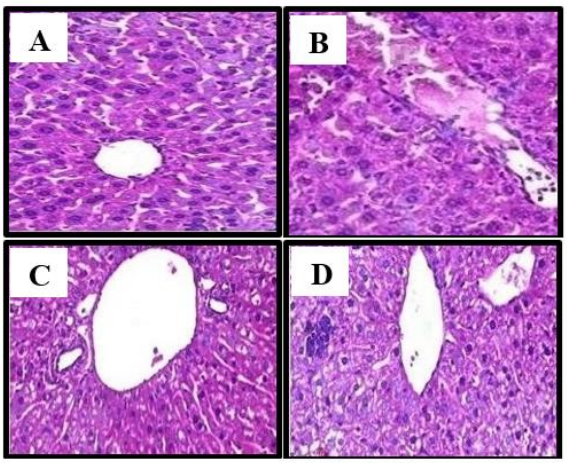

Figure 6: Liver sections photomicrographs (H&E, X 400) of normal control mice (A), EAC tumor-bearing mice (B), EAC tumor-bearing mice ingested silymarin orally (C), and EAC tumor-bearing mice ingested nano silymarin orally.

One animal cancer model with high malignancy and rapid growth resistance is Ehrlich ascites carcinoma. Silibin has been reported to be an antioxidant (Beydogan & Bolkent, 2016), and increase the activity of antioxidant enzymes (Altorjay et al., 1992; Gomes et al., 2008) which is consistent with our findings. They demonstrated how silibin affected the liver of mice harbouring Ehrlich ascites tumor (EAT) cells throughout a range of periods. The scientific method investigates possible natural immunomodulatory therapy and apoptotic processes to demonstrate how well nano silymarin overcomes the limitations or disadvantages of these useful drugs. According to Napolitano et al. (Napolitano et al., 2013), silymarin dramatically reduced the expression level of CXCR-4 in HepG2 cells and triggered apoptosis in a concentration-dependent manner. In recent experimental and clinical research, Fallah et al. (Fallah et al., 2021) demonstrated the effectiveness of silymarin in protecting the liver as well as against cancer *in vitro* and *in vivo*. This could be as a result of apoptosis being modulated by disrupting cell cycle regulator expression. The anti-inflammatory and anti-metastatic properties enhance the anti-cancer effects of radiation and chemotherapy in some cancer types.

4. CONCLUSION

Silymarin, a combination of active components from *S. marianum*, has been shown to have beneficial impacts on humans in both laboratory and clinical studies. Using HPLC, the present study has identified nano silymarin flavonolignan and flavonoid compounds and investigated their potential to reduce oxidative stress and repair damaged liver histoarchitecture in Ehrlich ascites carcinoma-bearing mice. After our investigation, we could propose that EAC tumor-bearing mice that induce tumor angiogenesis can be effectively employed as a model to demonstrate the potential impact of nano silymarin as a therapeutic target for related hepatic cancer.

5. CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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الملخص العربي

السيليمارين والنانو سيليمارين يخففان من الإجهاد التأكسدي والبنية التالفة للكبد في الفئران الحاملة لسرطان استسقاء إيرليش

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لقد استخدام نبات شوكه الجمل لعدة قرون لعلاج العديد من الأمراض. ويعتبر السليمارين هو المكون الحيوي الرئيسي الموجود في بذور هذ النبات. وبالتالي كان الهدف من هذه الدراسة هو تقييم قدرة السليمارين والنانو السليمارين على تخفيف الإجهاد التأكسدي وتحسين البنية النسيجية التالفة للكبد في الفئران المصابة بسرطان الاستسقاء إيرليش. لقد تم إجراء تحليل HPLC للمركبات الفلافونيدية الموجودة في السليمارين النانوي المستخلص من بذور نبات شوكة الجمل. ولقد تم تقسيم ٦٠ أنثى من الفئران السويسرية البيضاء بشكل عشوائي إلى أربع مجموعات متساوية. حيث استخدمت المجموعة الأولى كمجموعة ضابطة. في حين ان المجموعات الثلاث المتبقية فقد عوملت بتركيز ٢,٥ × ١٠^{٦} خلايا EAC داخل الغشاء البروتوني للبطن. كذلك لم تعامل المجموعة الثانية أي معاملات إضافية واستخدمت كمجموعة إيجابية ضابطة لـــ EAC. كذلك تم تجريع الفئران في المجموعتين الثالثة والرابعة عن طريق الفم بالسليمارين والنانو السليمارين بجرعة ١٠٠ مجم / كجم من وزن الجسم يوميًا لمدة ٦ أسابيع. ولقد تم تقييم علامات الإجهاد التأكسدي والأورام، وكذلك مضادات الأكسدة الأنزيمية وغير الأنزيمية، في جميع المجموعات التجريبية. علاوة على ذلك، فقد تم إجراء فحص نسيجي للكبد. أظهرت النتائج أن الفئران المصابة بسرطان الاستسقاء إيرليش كان لديها بنية نسيجية كبدية تالفة، ومستويات منخفضة من مضادات الأكسدة الأنزيمية وغير الأنزيمية، ومستويات عالية من علامات الورم والإجهاد التأكسدي. أدى إعطاء السليمارين والنانو السليمارين للفئران المصابة بسرطان الاستسقاء إيرليش إلى تقليل الإجهاد التأكسدي وتحسين البنية النسيجية للكبد التالف.