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STUDYING THE POSSIBLE EFFECT OF SILYMARIN AS A NATURAL EXTRACT AGAINST LEAD- INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

The aim of the current examination was scrutinizing the possible ameliorative impacts of silymarin against adverse effects and hepatotoxicity induced by lead toxicity in rats. Giving a daily basis dose of lead acetate (4mg/kg B.WT/ day/8 weeks) to rats caused oxidative liver injury which was shown by the increase in serum levels of hepatic markers enzymes (transaminases and gamma glutamyl transferase), inflammatory factors (tumor necrotic factor-alpha (TNF-α) and interleukin-6 (IL-6)) and the level of hepatic malondialdehyde (MDA) with marked severe damage in the histopathology of liver tissues compared with control rats. Also, lead significantly reduced the level of total antioxidant capacity (TAC), glutathione content (GSH) and the activity of superoxide dismutase activity (SOD) and catalase (CAT). While, the treatment of rats with lead along with silymarin (50mg/kg/day/8weeks) induced a significant decrease in the activity of liver enzymes, the level of TNF-α and IL-6 with enhancing the antioxidant status and reducing lipid peroxidation, as well silymarin treatment significantly reduced the severe damage and necrosis of liver tissues induced by lead. The results concluded that silymarin ameliorated the toxic effects of lead to a major extent, suggesting the probability of using silymarin as a powerful natural extract that can protect against liver injury.

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Introduction

World health organization revealed that several types of pathological disorders can be occurred because of pollution by toxic metals including lead [1]. Lead is widely used in the manufacturing of high-tech products for the nuclear reactorsprotection, thin sheets of electronic components, batteries, paints, ceramics and cables [2]. Toxicity of the body by lead would cause an excessive production of free radicals, oxidative damage and depletion of internal antioxidant defense systems. The extensive industrial use of lead has resulted in environmental pollutions, contamination of earth's crust and drinking water and various health hazards [2]. Also, this metal may have influences on the central nervous system, hematopoietic, hepatic and renal system and cause anemia, immunetoxicity and toxicity to the reproductive organs [1].

Natural herbs and plant extracts with high antioxidant contents can be used as therapeutic agents against hazards induced by exposure to toxic metals [3]. Herbs rich in natural antioxidants like Silybummarianum (Asteraceae), have been supposed to attenuate harmful effects of lead toxicity. Milk thistle (Silybummarianum) has been one of the most medicinal plants that can be used as a remedy for various hepatic disorders, including hepatitis and cirrhosis, and to prevent liver damage induced by chemicals and environmental toxins [4]. Silymarin is a natural extract isolated from the Silybummarianum and composed of a mixture of two different flavonoids (taxifolin and quercetin), and three flavonolignane diastereomers (Silibinin, silydianin and silychristen) [3]. Silymarin has been clinically used as cardioprotective, antidiabetic, antiinflammatory and heaptoprotective agent. The hepatoprotective activity of silymarin could be associated with some changes in its antioxidant activity, free radical scavenging and increasing endogenous antioxidant defense such as reduced glutathione [5]. Therefore, the current examination was done to estimate the impact of silymarin against liver damage caused by lead toxicity in rats.

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Material and Methods:

Silymarin powder, chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Determination of total phenol by Folin-Ciocalteu method

Total polyphenols were determined by Folin-Ciocalteu (FC) procedure [6]. Some aliquots of the extract (0.1 ml) were transferred into the test tubes, and the distilled water was added to make the volume up to 0.5 ml. After that, the tubes were vortexed after the addition of 0.25 ml Folin-Ciocalteu reagent (FCR) and 1.25 ml 20% aqueous sodium carbonate solution. Finally, the absorbance of the blue color of the mixtures was recorded at 765 nm after 120 min. From the calibration curve of Gallic acid standard solutions (covering the concentration range between 0.1 and 1.0 mg/ml), the total amount of polyphenols was calculated as a Gallic acid equivalent, and the weight of Gallic acid was determined in mg per mg dry material. All experiments were conducted in triplicate and the values were indicated as the mean ± SD.

Ferric Reducing/Antioxidant Power (FRAP) assay

For this investigation, the FRAP assay reported by Benzie and Strain (1996) was applied, and a simple and reliable test was adopted depending upon the reduction of ferric [Fe (III)-TPTZ] to [Fe(II)- TPTZ] complex by a reductant at low pH, was adopted [7]. This complex had an intense blue color that could be detected at 593 nm. Assays were performed by adding 1.45 ml of FRAP solution and 0.05 ml of 0.1 mg/ml silymarin.

The absorbance was recorded at 593 nm. All experiments were done in triplicate, and the values were expressed as the mean ± SD.

Drug and dosage:

Pure powder of silymarin was procured from Sigma Chemicals. Silymarin powder was administered orally to the animals at therapeutic dose 50mg/kg after dissolving the powder in distilled water [8].

Experimental Protocol:

Animals

Male albino rats (150 ± 20 g) were kept in the controlled laboratory conditions for two weeks for acclimation. They were fed on stock rodent diet and tap water that were provided to them ad libitum. The animals were treated based on the principles listed in the NIH Guide for the care and use of laboratory animals.

Grouping of animals

The rats were randomly divided into 4 groups, each group included 7 rats. Control group: The rats were fed on balanced diet and served as control.

Silymarin group: The rats were fed on balanced diet and orally received silymarin (50mg/kg/day) for 8weeks.

Lead acetate treated group (Pb group): a dose of 4mg/kg B.WTlead acetate was given to the rats [9] by orogastric tube for 8weeks.

Pb + Silymarin group: The rats received lead acetate (4mg/kg B.WT) along with silymarin (50mg/kg/day) for 8 weeks.

At the end of the experiment, 24 hrs post the last dose of treatment, the animals from each group were sacrificed. Using diethyl etherthe, the rats were anathesized, and the blood samples were withdrawn by cardiac puncture and allowed to coagulate.The serum was collected using the centrifuge for biochemical analysis.

Moreover, the liver tissue was removed, and saved for biochemical investigation.

Biochemical Analysis

The activity of serum alanine transaminase (ALT) and aspartate transaminase (AST) was estimated according to Reitman and Frankel (1957) and serum gamma glutamyl transferase (GGT) was determined according to Rosalki (1975) [10, 11]. ELISA technique (BioSource International,Camarillo, CA, USA) was applied for identifying serum tumor necrotic factoralpha (TNF-α) and interleukin-6 (IL-6) based on the manufacturer's instructions.

Liver was dissected, completely washed with ice-cold 0.9% NaCl, and weighed, using 66 mmol/L chilled phosphate buffer (pH 7.0) the liver was minced and homogenized (10% w/v). The homogenates then were centrifuged at 6000 rpm for 15 min, and the supernatants were gathered to assess malondialdehyde (MDA) [12], total antioxidant capacity (TAC) [13], glutathione content (GSH) [14], superoxide dismutase activity (SOD) [15] and Catalase activity (CAT) [16].

Histopathological Examination

Finally, the tissue samples were rapidly taken from each rat for histopathological study, and fixed in 10% formalin. In ascending grades of ethanol, all the samples were dehydrated, then cleared in butanol, and embedded in parablast. Thick sections of 5-6 µm were obtained, and stained with the following stains:

1. Haematoxylin and Eosin (H&E) staining for general histological studies.
2. Masson's Trichrome stain for collagen fibers.

Statistical analysis

The results were presented as mean ± SE (n = 7). The experimental data were analyzed using one way analysis of variance (ANOVA). Duncan's multiple range test was used to determine the significant differences between the means. Statistical analyses were performed using Statistical Packages for Social Science [17]. The differences between the means were considered significant at $P < 0.05$.

Results:

Total phenol content of silymarin was 0.535±0.019 mg Gallic acid equivalent (GAE) / mg; and total antioxidant capacity of silymarin (0.1 mg/ml) was 142±10.8µmol/l (Table 1).

Table 1: Evaluation of total phenol contents and antioxidant capacity of silymarin

Parameters	SilymarinMean ± S (n=3)
Total phenol content (mg GAE /mg)	0.535±0.019
Total antioxidant activity (µmol/l)	142±10.8

Comparing to the control group, the results showed that the injection of lead to rats induced an obvious elevation in the activity of liver enzymes (ALT, AST and GGT), TNF-α and IL-6 levels and hepatic MDA with significant reduction in the level of TAC and GSH content and SOD and CAT activity of liver tissues of lead-intoxicated rats (Table 2).

Table 2: Effect of silymarin administration on the serum activities of ALT, AST and GGT in rats treated with hepatotoxic dose of lead acetate.

Parameters	Control	silymarin	Pb	Pb+silymarin
AST (U/ml)	37.45 ± 1.28^{c}	35.66 ± 1.53^{c}	63.65 ± 2.78^{a}	42.12 ± 1.92^{b}
ALT (U/ml)	26.36 ± 0.90^{c}	25.14 ± 0.82^{c}	40.15 ± 1.03^{a}	31.44 ± 0.85^{b}
γGT (U/ml)	4.25 ± 0.38^{c}	4.19 ± 0.45^{c}	6.73 ± 0.59^{a}	5.52 ± 0.64^{b}

Means in the same row with different superscripts are significantly different at ($P<0.05$), Values are expressed as mean ± S.E. (n=7).

On the other hand, the concomitant administration of silymarin with lead acetate resulted in a significant reduction in the activities of serum liver enzymes and serum levels of inflammatory cytokines (TNF-α and IL-6), as well, silymarin caused the marked activation of antioxidant enzymes and increased the level of total antioxidant capacity with reduction in hepatic MDA when compared to the group of rats injected with lead acetate only (Table 3 & 4).

Table 3: Effect of silymarin administration on TNF-α and IL-6 levels in rats treated with toxic dose of lead acetate.

Parameters	C	silymarin	Pb	Pb+silymarin
TNF-α (pg/mL)	687.56 ± 60.10^{c}	674.98 ± 62.35^{c}	912.15 ± 86.28^{a}	746.83 ± 61.56^{b}
IL-6 (pg/mL)	341.26 ± 25.45^{c}	336.67 ± 26.27^{c}	508.86 ± 45.65^{a}	382.38 ± 35.48^{b}

Means in the same row with different superscripts are significantly different at ($P<0.05$), Values are expressed as mean ± S.E. (n=7).

Table 4: Effect of silymarin administration on hepatic MDA and antioxidant status in rats treated with toxic dose of lead acetate.

Parameters	Control	Silymarin	Pb	Pb+silymarin
MDA (n mol/g tissue)	174.24 ± 4.32^{c}	170.64 ± 4.28^{c}	312.16 ± 7.18^{a}	210.15 ± 4.72^{b}
TAC (U/mg protein)	0.74 ± 0.05^{a}	0.75 ± 0.04^{a}	0.47 ± 0.04^{c}	0.66 ± 0.05^{b}
GSH (mg/g tissue)	28.39 ± 2.62^{a}	28.86 ± 2.47^{a}	18.83 ± 1.73^{c}	24.88 ± 1.32^{b}
SOD (U/mg protein)	48.96 ± 3.96^{a}	49.35 ± 3.67^{a}	27.72 ± 2.90^{c}	41.75 ± 3.63^{b}
CAT (U/mg protein)	54.46 ± 1.68^{a}	54.92 ± 1.93^{a}	31.97 ± 1.70^{c}	48.82 ± 1.68^{b}

Means in the same row with different superscripts are significantly different at ($P<0.05$), Values are expressed as mean ± S.E. (n=7).

Histopathology results:

The histopathological examination of the liver sections of control and other rats groups has been demonstrated in Figure 1. The control rats showed liver tissues with normal architecture, normal central vein without damage with no change in sinusoids and hepatocytes architecture (A). The same normal architecture was shown in the histological structure of liver sections of rats treated with silymarin (B). The liver sections of rats intoxicated with Pb showed necrosis in hepatic cells, vacuolated hepatocytes with severe

damage associated with central vein (C). The treatment of rats with Pb and silymarin appeared to significantly reduce the severity of damage and minimal necrosis, and showed regeneration of hepatocytes (D).

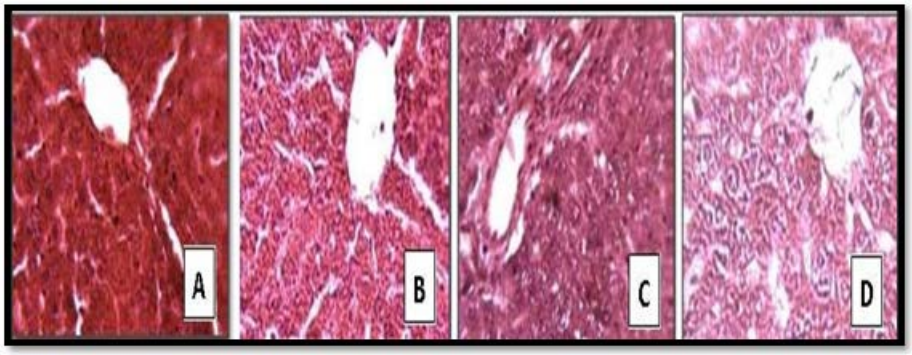

Figure 1: Light photomicrograph of liver sectional histology from control rats (A), silymarin (B), lead (C) and Lead+Silymarin (D) (H&E. X 400).

Discussion:

Exposure to industrial pollutants and toxic chemicals like lead can cause oxidative stress and occurrence of liver injury [18]. Silymarin is one of dietary antioxidants that can play an effective role against free radicals and reduce the oxidative damage [4]. In this work, the recorded results of total phenolic content (0.535±0.019 mg GAE/ mg) and total antioxidant capacity (142±10.8µmol/l) of silymarin revealed that this natural extract possessed high antioxidant capacity and can be applied as a strong antioxidant herbal drug which can preserve biological systems in case of the oxidative stress [19].

According to the results of liver enzymes in this study, lead caused a remarkable elevation in the activity of liver transaminases indicating that lead has hepatotoxic effects [20]. The rises in the liver enzymes could be due to hepatic cellular damage induced by lead toxicity resulting in change in cell permeability and release of cytosol from the liver into the blood stream [21]. Todorovic et al. (2005) suggested that the accumulation of lead in liver would lead to the destruction of permeability of hepatocytes cell membrane and leakage of liver enzymes into the serum [22].

On the other hand, activities of liver enzymes of rats treated with Pb and silymarin were lower than those of rats treated with Pb only. The antihepatotoxic effect of silymarin can be due to the point that it can prevent cellular leakage and loss of functional integrity of the cell membrane in liver [23]. It has been suggested that silymarin has vital therapeutic applications for repairing the damaged hepatocytes and restoration of normal liver functions by acting on RNA polymerase enzymes and increasing ribosomal formation which in turn hastens protein and DNA synthesis [24].

The results revealed that Pb administration caused marked increase in the level of TNF-α and IL-6 indicating the occurrence of inflammation which were consistent with the previous report of [25]. A study of Marwa and Hassanein (2013) showed that lead intake caused remarkable increases of interleukin-6 (IL-6) with induction of inflammation and tissue damage in heart degenerative cells and serum of rats treated with lead [26]. Pb might increase the transcription of TNF-α mRNA in hepatocytes [27].

In the present study, the intake of silymarin along with Pb effectively reduced the increases in the level of TNF-α and IL-6 suggesting antiinflammatory effect of silymarin [28]. These findings could be attributed to the ability of silymarin to inhibit nuclear factor-kappa B (NF-kB) action which has been known to be one of the critical transcription factors required for maximal transcription of a wide array of proinflammatory molecules, including TNF-α, IL-1b, IL-6 and other mediators [29].

In the present work, the exposure of rats to Pb resulted in increased level of hepatic MDA with reduction of TAC, GSH and the activity of SOD and CAT. The damage effect of lead could be related to overproduction of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxides, evaluated by MDA levels as the final products of lipid peroxidation [30]. Also, Pb caused the inactivation of antioxidant enzymes by binding to the sulfhydryl group of enzymes of the anti-oxidative defense system and replacing divalent bioelements that serve as important co-factors of antioxidant enzymes [31].

On the other side, treatment of rats with silymarin and Pb induced the significant activation of antioxidant enzymes with reduction of MDA compared to rats treated with Pb only. Various studies indicated that silymarin had strong antioxidant activity and can protect against liver toxicity induced by a toxic agents by inhibiting lipid peroxidation [32]. The hepatoprotective effect of silymarin could be related to its ability to scavenge free radicals as well as increase the cellular content of GSH [33]. Yaman et al. (2018) reported that silymarin treatment seemed to have protective effects against methotrexate toxicity by enhancing the activities of antioxidant enzymes and decreasing MDA levels due to its bioactive chemical components including flavolignans, silydianin, silychristin, and silybin [34].

Conclusion:

The results of this study supported that silymarin has a protective role against lead toxicity as it reduced the activity of liver enzymes, inflammatory factors and MDA, and enhanced the antioxidant status with a significant reduction of severe damage of liver tissues induced by lead toxicity. Thus, silymarin can be used as a natural extract possessing high antioxidant potential against toxic agents.

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