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Attenuation of Benzo[*a*]Pyrene-Induced Oxidative Stress and Cell Apoptosis in Albino Rats by Wild Milk Thistle (*Silybum Marianum* L.) Seeds Extract

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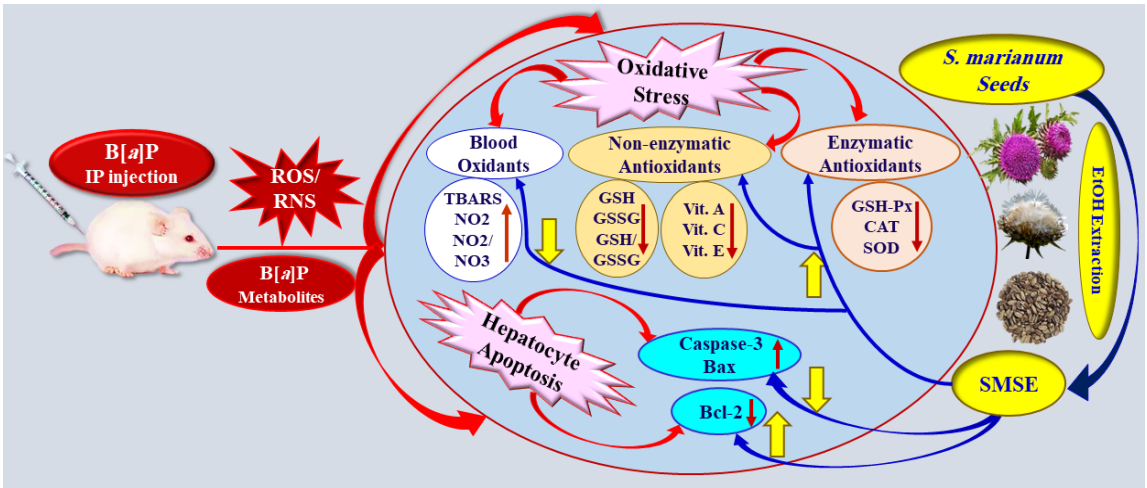
Abstract

Oxidative stress (OS) is considered a pathogenic mechanism that, if not strictly controlled, triggers the onset and progression of many chronic and degenerative diseases. This investigation aimed to explore the potential attenuation of OS and cell apoptosis induced by benzo[*a*]pyrene (B[*a*]P) in albino rats using wild *Silybum marianum* seed ethanolic extract (SMSE). Thirty-six adult male albino rats were divided into six equal groups. Group (1) served as the normal control and received an intraperitoneal vehicle of single-dose corn oil (10 ml/kg/b. wt.). The remaining groups (2-6) were subjected to a single dose of B[*a*]P (125 mg/kg/b. wt.) dissolved in corn oil. Subsequently, group (2) was assigned as the positive control, while the other groups (3 to 6) received SMSE at doses of 200, 400, 600, and 800 mg/kg/d, respectively, by oral gavage for four weeks. B[*a*]P administration resulted in severe OS as evidenced by a significant ($p \leq 0.05$) increase in plasma oxidants levels (thiobarbituric acid reactive substances TBARS, No_2, and No_2/No_3); and a marked drop in the levels of erythrocyte enzymatic antioxidants (glutathione peroxidase GSH-Px, catalase CAT, and superoxide dismutase SOD), non-enzymatic antioxidants (reduced glutathione GSH and oxidized glutathione GSSG) and plasma antioxidant vitamins (A, C, and E). Additionally, B[*a*]P exposure activated liver apoptosis by upregulating caspase-3 (a cell apoptosis executor) and Bax (a pro-apoptotic protein); and downregulating Bcl-2 (an anti-apoptosis marker). Treatment with SMSE markedly attenuated OS by suppressing plasma oxidants or restoring enzymatic and non-enzymatic antioxidant armories in a concentration-dependent manner. Moreover, the apoptotic proteins caspase-3 and Bax were inhibited, whereas the anti-apoptotic mediator Bcl-2 was activated. In conclusion, the obtained data provide potent proof of SMSE's efficiency as a preventative and curative natural remedy against B[*a*]P-induced oxidative stress damages.

Keywords: *Silybum marianum* seeds; oxidative stress; blood oxidants; enzymatic antioxidant; glutathione fractions; antioxidant vitamins; hepatic apoptosis; benzo[*a*]pyrene.

Graphical abstract



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1. Introduction

Free radicals (FRs) are extremely dangerous atoms or molecules of reactive species derived from oxygen and nitrogen that created by a variety of endogenous and exogenous processes [1]; the endogenous factors may include respiration, digestion, drug metabolism, fat oxidation, immune cell activation, inflammation, psychological stress, infection, cancer, and aging whereas, the exogenous factors involve environmental pollutants exposure, heavy metals, drugs, smoke, alcohol and radiation [2, 3]. Oxygen free radicals, including superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), lipid peroxide ($LOO^{\bullet}$) radicals, hydroxyl radicals ($^{\bullet}OH$), and singlet oxygen ($^1O_2$), are commonly referred to as reactive oxygen species (ROS), which are produced during the metabolism process of oxygen [4]. Reactive nitrogen species (RNS), including nitrogen dioxide (NO_2), nitric oxide ($^{\bullet}NO$) radicals, and peroxynitrite ($ONOO^-$), are generated from nitric oxide (NO) and ($O_2^{\bullet-}$) by inducible nitric oxide synthase (iNOS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, respectively [5]. FRs and associated molecules are typically classified as reactive oxygen species (ROS), reflecting their propensity to induce oxidative disorders within the cell [6]. As a result of the unpaired electrons in their structure, ROS are extremely reactive molecules that interact with various cellular biological macromolecules, including nucleic acids, proteins, lipids, and carbohydrates, altering their functioning [7].

The body has a complex antioxidant system that restricts the detrimental effects of FRs and counteracts excessive FRs generation. Several endogenous and exogenous antioxidants protect the body from oxidative damage [8]. ROS is a double-edged sword. At the physiological level, ROS defends the body from pathogens like microbes, acting as an immune system to fight off foreign substances [9, 10]. Physiologically, a limited amount of ROS is generated intracellularly, acts in cell signaling and can be rapidly decreased by the antioxidant defenses. However, under pathological and stressful conditions, ROS generation surpasses the neutralization capacity of the antioxidant defense system, creating a condition called ‘‘oxidative stress’’ OS [11, 12]

OS is an imbalance between both the generation and accumulation of ROS in tissues and cells and the capability of a biological system to eliminate such reactive products or repair oxidative damage [13]. OS plays a pivotal role in the pathogenesis of chronic illnesses like cardiovascular disease, diabetes, chronic obstructive pulmonary disease, neurological disorders, reproductive dysfunction, chronic kidney disease, obesity, cardiac hypertrophy, and cancer [1, 14].

Prolonged exposure to pro-oxidant agents could induce severe damage to cell membranes, inhibit cellular processes needed to maintain body functioning, disrupt normal cell division, and structural flaws in mitochondrial DNA along with functional changes in numerous enzymes and biological structures [15, 16]. Moreover, OS can exacerbate multiple sclerosis, Alzheimer's disease, and Parkinson's disease. The accumulation of FRs in the body can lead to cognitive impairment, dementia, and an elevated risk of stroke [6, 17].

Plant-derived bioactive compounds have attracted much interest over the last few decades due to their therapeutic use in treating and preventing disease, using whole plants, plant extracts, or isolated components with complete phytochemical profiles [16]. *Silybum marianum* SM, commonly known as milk thistle, is a well-known medicinal plant of the genus *Silybum* a member of the *Asteraceae* family, native to the Mediterranean region that contains silymarin, the main ingredient, and is proven to treat a variety of physiochemical diseases [18, 19, 20]. SM seeds and fruits have been used as a natural treatment for over 2000 years due to their hepatoprotective properties [21]. SM possesses antibacterial, anticancer, hepatoprotective, cardiovascular-protective, neuroprotective, skin-protective, and anti-diabetic properties [22]. The silymarin extract from milk thistle is a complex combination of plant-derived components, primarily flavonolignans, flavonoids (taxifolin, quercetin), and polyphenolic compounds [23]. Silymarin contains four major flavonolignan isomers: silibinin, isosilibinin, silichristin, and silidianin. Silibinin (also called silybin) is the most abundant and biologically active. Silibinin accounts for around 50-60% of the silymarin mixture, and the other flavonolignan isomers account for approximately 35%. These components are widely known for their antioxidant properties, in addition to possessing a wide range of other biological functions [24].

Natural antioxidants are becoming a focus of sophisticated scientific research. In this context, and in an attempt to discover new avenues for the exploitation of available natural resources to counteract the destructive effects of OS, this study planned to evaluate the potential attenuation of benzo[*a*]pyrene-induced OS and cell apoptosis in adult albino rats by wild milk thistle (*Silybum marianum* L.) seeds extract.

2. Materials and methods

2.1. Materials

2.1.1. Silybum marianum seeds (SMS)

A healthy mature wild milk thistle (*Silybum marianum*) sample was gathered from Mit Ghorab Village, Senbellawein Center, Dakahlia Governorate,

Egypt. The sample has been botanically authenticated by plant taxonomy specialists at the Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt. The seeds were removed from dry fruits, purified of exterior matter, dried in the shade at room temperature to prevent photo-oxidation, and kept in sealed glass containers until use.

2.1.2. Reagents and chemicals

B[*a*]P ($C_{20}H_{12}$); standards for vitamins (A, C, and E); and thiol compounds (GSH and GSSG) were acquired from Sigma Chemical Co., St. Louis, MO, USA. While Morgan Chemical Company, Cairo, Egypt supplied the basal diet constituents. The remaining chemicals, reagents, and solvents were of analytical quality and obtained from El-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Instruments Trading Co. in Cairo, Egypt.

2.1.3. Experimental animals

The experiments were performed on ten-week-old adult male albino Sprague-Dawley rats (170-185 g) obtained from the Laboratory Animal Unit, College of Veterinary Medicine, Cairo University, Egypt. The rats were kept in stainless steel cages under typical laboratory conditions (~22 °C temperature, 60-70% relative humidity, and a 12/12 hour light-dark cycle). The rats were acclimated for a week prior to the study, fed on a basal diet, and had free access to water throughout the study. All biological experiments were carried out according to the guidelines for the care and use of laboratory animals established by the Institutional Animal Care and Use Committee (IACUC), Menoufia University, Sheibin El-Kom, Egypt. The IACUC ethically approved all experiments for this work (Approval No., MUFHE /F/NFS/14/23).

2.2. Methods

2.2.1. Silybum marianum seeds ethanolic extract (SMSE)

SMSE was prepared from dried SMS, which were crushed into a fine powder with an electric mixer. The extraction process was conducted for five days in absolute ethanol (1: 10 w/v) in a flask shielded from light at 25°C with periodic shaking. Following maceration, the extracted content was filtered using Whatman (No.1) filter paper to separate the filtrate from the residues. The filtrates were then evaporated to a constant weight using a Rotary evaporator (Heidolph VV 2000) at 40 °C and 50 rpm under low pressure, as described by Javeed *et al*. [20]. The extract was kept at 4°C until use.

2.2.2. Basal diet

The basal diet was prepared from fine ingredients in accordance with Reeves *et al*. [25]. The composition of the basal diet consisted of corn oil (10%), protein (10%), mineral mixture (4%), vitamin mixture (1%), methionine (0.3%), cellulose (5%), choline chloride (0.2%), and the remaining constituent was corn starch (69.5%).

2.2.3. Experimental design

Following the 7-day acclimatization period, thirty-six adult male albino rats were randomly divided into six groups, each comprising six rats. Group (1) served as the normal control group and received an intraperitoneal vehicle of a single dose of corn oil (10 ml/kg/b. wt.). The remaining groups (2-6) were injected with a single dose of B[*a*]P (125 mg/kg/b. wt. dissolved in corn oil) via intraperitoneal injection. Subsequently, group (2) acted as the positive control group, while the other groups (3 to 6) were administered SMSE at concentrations of 200, 400, 600, and 800 mg/kg/d, respectively, by oral gavage for 28 days. Throughout the experiment, all animals were fed on the basal diet and allowed access to water *ad libitum*.

The selection of treatment dosages of SMSE to alleviate oxidative stress and cell apoptosis induced by B[*a*]P was based on previous studies [26, 27]. Moreover, the B[*a*]P dose was selected in accordance with Shahid *et al*. [28].

2.2.4. Biological evaluation

Throughout the experiment, feed intake (FI) was measured daily and body weight was reported weekly. Body weight gain (BWG %) and feed efficiency ratio (FER) were estimated in accordance with Chapman *et al*. [29].

2.2.5. Blood and liver tissues sampling

At the end of the experiments, the rats were slaughtered under sodium pentobarbital anesthesia after 12 hours of fasting. Blood samples were obtained through the abdominal aorta and placed in centrifuge tubes with oxalate solution (1.34%) as an anticoagulant. Plasma was carefully separated after centrifugation at 4000 rpm for 10 minutes to assess the levels of glutathione fractions, oxidants, and antioxidant vitamins. The erythrocyte residue was rinsed three times with 0.9% NaCl solution before being hemolysin with deionized water for 30 minutes. The hemolysate was further centrifuged for 30 minutes at 30,000 rpm. The supernatant was used to determine the levels of enzymatic antioxidants in accordance with Stroev and Makarova [30]. The liver samples were obtained and promptly snap-frozen in liquid nitrogen before being kept at -80 ^{o}C for molecular analysis of apoptotic markers.

2.2.6. Plasma oxidants assay

Blood oxidants concentration, including thiobarbituric acid reactive substances (TBARS), were measured as described by Buege and Aust [31]; nitrite NO_2, as well as nitrite/nitrate NO_2/NO_3 levels, were determined according to the method of Misko *et al*. [32].

2.2.7. Non-enzymatic antioxidants assay

Reduced and oxidized glutathione (GSH and GSSG) contents in plasma were estimated by HPLC following the method described by McFarris and Reed [33]. While plasma levels of antioxidant vitamins A, C, and E were determined as prescribed by Epler *et al.* [34], Moeslinger *et al*. [35], and Hung *et al*. [36], respectively.

2.2.8. Enzymatic antioxidants assay

Erythrocytes' enzymatic antioxidant activities, including superoxide dismutase SOD, glutathione peroxidase GSH-Px, and catalase CAT were estimated following the procedures described by Marklund, S. and Marklund, G. [37], Splittgerber and Tappel [38], and Aebi [39], respectively.

2.2.9. Liver cell apoptosis assays

Liver samples were prepared and analyzed for B-Cell Lymphoma 2 (Bcl-2), Bcl-2-like Protein 4 (Bax), and caspase-3 using a flow cytometer instrument (Becton Dickinson, San Jose, CA, USA) according to the method of Gong *et al*. [40].

2.3. Statistical analysis

The data collected were statistically analyzed using SPSS V22 software. The results were presented as mean ± S.D. One-way analysis of variance (ANOVA) and Duncan's post hoc test were used to compare differences between groups. Statistical significance was determined at $P \leq 0.05$ for all comparisons.

3. Results and discussion

3.1. Effect of SMSE on BWG, FI and FER of B[a]P-treated rats

Throughout the experiment, we kept track of each group's body weight gain (BWG), feed intake (FI), and feed efficiency ratio (FER). Figure (1) illustrates the effect of SMSE on BWG, FI, and FER of B[*a*]P-treated rats. As compared to the normal control group, it was clear that B[*a*]P treatment caused a significant ($p \leq 0.05$) decrease in BWG, FI, and FER in the positive control group by the ratios of (-46.07, -34.09, and -30.99%), respectively. On the other hand, SMSE intervention in B[*a*]P-treated rats prevented these variables from declining and partially restored their rates to normal levels, However, these changes did not reach the significant level in terms of SMSE-treated groups (200 and 400 mg/kg/d), whereas both SMSE-treated groups (600 and 800 mg/kg/d) exhibited a significant ($p \leq 0.05$) increase in BWG and FI values when compared to the positive control group.

These findings are compatible with those found by Elhassaneen *et al*. [41], who observed that injection of B[*a*]P at a dose of 15 mg/5 ml/kg b. wt. dissolved in 0.9% NaCl solution in male albino rats led to a decrease in body weight compared with normal rats. Also, Tag Al Deen and Ghozy [42] noted a marked decrease in BWG and FI by (-67.10%, -23.70%), respectively, in rats injected with one milliliter of a carcinogenic solution (10.08 mg/kg) containing B[*a*]P compared to normal rats. In contrast, Yang *et al*. [43] reported that treatment of male Wistar rats (6 weeks old) with a single oral dose of B[*a*]P (25 mg/kg b. wt. in corn oil) had no impact on body weight. In addition, Kumar *et al*. [44] demonstrated that oral administration of 50 mg/kg B[*a*]P twice weekly in olive oil for four weeks resulted in no significant differences in body weight in experimental rats. These variances might be attributed to differences in B[*a*]P dosage concentration, administration route, and experimental duration.

The obtained data showed that SMSE treatment at high concentrations resulted in a significant improvement in FI, BWG, and FER than at low SMSE concentrations in B[*a*]P-treated rats. Talbi *et al*. [45] observed that *S. marianum* seeds-infused solution (200 mg/kg/day) exhibited no significant difference between all groups of ethanol-induced reprotoxicity in male rats in terms of body weight gain. Furthermore, Aghazadeh *et al*. [46] reported that administering *S. marianum* extract at a dosage equivalent to 1.0 g seeds powder/kg b. wt./d for three weeks had no significant effect on food consumption and body weight among nonalcoholic steatohepatitis-treated rats. The improvement in BWG, FI, and FER of B[*a*]P-treated rats by SMSE at high concentrations may be due to its high antioxidant capacity, which scavenges free radicals and alleviates oxidative stress induced by B[*a*]P.

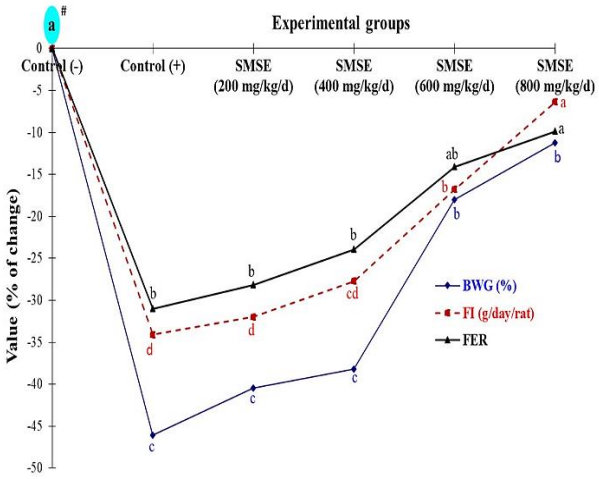

Figure 1. Effect of SMSE on BWG, FI and FER of B[*a*]P-treated rats (as % of the normal control).
 # Different letters for each variable indicate a significant difference (P≤0.05).

3.2. Effect of SMSE on blood oxidants concentration of B(a)P-treated rats

To investigate the effect of SMSE on OS status in B[*a*]P-treated rats, the plasma oxidants concentration, including thiobarbituric acid reactive substances TBARS (a marker of OS-induced lipid peroxidation);

nitrite NO_2; and nitrite/nitrate NO_2/NO_3 (products of NO oxidation by $O_2^{\bullet-}$, which serve as reliable indicators for evaluating of unstable ROS) were assessed and presented in Table (1). According to the data, intraperitoneal injection of B[*a*]P induced a significant ($p \leq 0.05$) elevation in TBARS, NO_2, and NO_2/NO_3 concentrations by 105.26, 104.15 and 65.74%, respectively, in the positive control group compared with the normal control. Oral administration of SMSE attenuated the accumulation of blood oxidants, as the groups treated with SMSE at doses (600 and 800 mg/kg/d) showed a significant ($p \leq 0.05$) decrease in TBARS and NO_2 levels as compared to the positive control group, with rates of change of (-33.3 and -43.59%); and (-31.50 and -41.80%), respectively. In contrast, SMSE (200 and 400 mg/kg/d) showed no significant differences. Also, it was observed that the mean values of NO_2/NO_3 in all SMSE-treated groups were lower than those of the control (+Ve) but failed to reach a significant level except for SMSE (800 mg/kg/d). Interestingly, SMSE (800 mg/kg/d) exhibited the most attenuating effect on plasma oxidants accumulation, while SMSE (200 mg/kg/d) had the least. The current findings are consistent with earlier studies that indicated that B[*a*]P exposure induced a significant increase in OS biomarkers such as lipid peroxidation (LPO), NO_2, NO_2/NO_3 as well as protein carbonyl contents PCC in experimental animals [44, 47, 48, 49].

Table 1
Effect of SMSE on blood oxidants concentration of B[*a*]P-treated rats

	Normal control	Control (+Ve)	SMSE (mg/kg/d)-Treated Groups				L.S.D
			200	400	600	800	
Thiobarbituric acid reactive substances (TBARS, nmol/mL)							
Mean	0.19^{d}	0.39^{a}	0.31^{abc}	0.33^{ab}	0.26^{bcd}	0.22^{cd}	0.091
SD	0.01	0.09	0.04	0.04	0.06	0.03	
% of change	0.00	105.26	-20.51	-15.38	-33.33	-43.59	
Nitrite (NO_2, nmol/L)							
Mean	2.41^{e}	4.92^{a}	4.58^{a}	4.11^{b}	3.37^{c}	2.86^{d}	0.409
SD	0.12	0.19	0.05	0.35	0.35	0.14	
% of change	0.00	104.15	-6.84	-16.53	-31.50	-41.80	
Nitrite/Nitrate (NO_2/NO_3, nmol/L)							
Mean	4.32^{c}	7.16^{a}	6.38^{ab}	6.20^{ab}	5.94^{ab}	5.57^{bc}	1.29
SD	0.84	1.03	0.64	0.78	0.60	0.10	
% of change	0.00	65.74	-10.89	-13.41	-17.04	-22.21	

Means in the same row with different superscript letters are significantly different at $p \leq 0.05$

B[*a*]P is a polycyclic aromatic hydrocarbon (PAH). B[*a*]P is one of the most toxic environmental contaminants, with several adverse consequences for human health [50, 51, 52]. B[*a*]P has been experimentally proven to induce OS in erythrocytes, blood monocytes, and other tissues [48, 53, 54, 55]. PAHs, such as B[*a*]P, are converted into specific phenols (hydroxy derivatives), phenolic diols, dihydro diols, quinones, and epoxide enantiomers of reactive diols. ROS are formed as byproducts of PAHs transformation [50, 56]. Additionally prostaglandin H synthase and lipoxygenase are also involved in the B[*a*]P metabolic transformation, which leads to the generation of highly reactive oxygen free radicals [57]. According to Penning *et al*. [58] the metabolites of B[*a*]P, such as quinines and phenols, are critical components of the redox cycle that occurs during B[*a*]P metabolic processes via one-electron oxidation followed by a nucleophilic attack by water to create 6-hydroxy-B[*a*]P. The 6-Hydroxy-B[*a*]P is unstable and quickly radicalizes to 6-oxo-B[*a*]P. After its formation, this radical reacts with cellular macromolecules or undertakes further interactions with molecular oxygen, leading to the quick generation of quinones [50, 59]. Quinones are not inert and therefore react actively with nucleic acids or exhibit cytotoxic or mutagenic impacts via superoxide anion radicals $O_2^{\bullet-}$ produced through the redox cycle [60]. Furthermore, Verma *et al*. [61] reported that B[*a*]P and its metabolites are proven to be toxic by disrupting cellular signaling pathways and inducing OS, resulting in abnormalities in target cell structure and function.

Our results revealed that B[*a*]P induced an overabundance of plasma oxidants that overpowered the antioxidant system to neutralize them, resulting in the development of OS. Lipid peroxidation (LPO) is a biomarker to assess OS. LPO levels rise rapidly during OS because FRs generate peroxidative damage to polyunsaturated lipid membranes. The severity of OS

is demonstrated by the levels of LPO products, specifically malondialdehyde (MDA), a reactive component of thiobarbiturate, which accumulates as the end product of LPO and serves as an indicator of the extent of OS [62]. Excessive hydroxyl radicals ($^{\bullet}OH$) and peroxynitrite ($ONOO^{-}$) can trigger LPO and damage cell membranes and lipoproteins, leading to the formation of MDA, which is cytotoxic and mutagenic [63]. Also, excessive NO interacts with the superoxide anion $O_2^{\bullet-}$ to create the $ONOO^{-}$ radical. This radical induces severe cellular damage via oxidizing and nitrating cellular macromolecules. In addition, elevated NO depletes reduced glutathione GSH, a non-enzymatic antioxidant (see Table 2), and rendering cells more vulnerable to OS [64].

On the other hand, treatment with SMSE dramatically reversed these changes by attenuating the elevation of blood oxidants induced by B[*a*]P, indicating that SMSE may have the potential to offer protection against OS. These findings were consistent with Serçe *et al*. [65] who found that milk thistle seeds (MTS) extract markedly suppressed TBARS generation and reported that MTS exhibits considerable DPPH (2, 2-diphenyl-1-picrylhydrazyl)-free radical scavenging activities and blocks lipid peroxidation. Moreover, Aghazadeh *et al*. [46] demonstrated that *S. marianum* (SM) extract significantly decreased hepatic MDA levels in experimental nonalcoholic steatohepatitis by 40%. Also, Abd Elalal *et al*. [66] discovered that the administration of ethanolic extract of *S. marianum* seed in busulfan-treated rats resulted in a substantial ($p \leq 0.05$) drop in MDA levels in all organ tissues, with the most notable decrease observed in the liver, testis, and spleen.

Silymarin (a plant-derived flavonoid that acts as a natural antioxidant) is a flavonolignan and one of the most bioactive components extracted from the fruits and seeds of SM [67]. Silymarin is a significant component found primarily in SM's leaves, fruit, seeds, and roots, with mature seeds containing a higher concentration of silymarin [68]. Silymarin's potential antioxidant mechanisms may involve (a) preventing the generation of free radicals by ROS-producing enzymes; (b) direct elimination of the effects of free radicals; (c) promoting the production of protective molecules that protect against the stress stimulus [24]; and (d) activation of enzymatic antioxidants like SOD and non-enzymatic pathways, primarily through Nuclear factor-erythroid factor 2 (Nrf2) activation [69].

Moreover, Haddadi *et al*. [62] found that silymarin inhibited lipid peroxidation and improved OS in 6-hydroxydopamine (6-OHDA). It is known that 6-OHDA generates free radicals that induce OS in intoxicated rats. This finding suggests that silymarin's antioxidative stress activity was attributable to the enhancement of the antioxidant defense system, as well as the inhibition of free radical generation.

3.3. Effect of SMSE on glutathione molecules homeostasis in B[a]P-treated rats

Glutathione is an endogenous non-enzymatic antioxidant that exists in two forms: reduced (GSH) and oxidized (GSSG). Glutathione acts as an antioxidant and protects cells from free radical damage. Table (2) reflects the effect of SMSE on plasma glutathione molecule levels (GSH and GSSG) and GSH/GSSG ratio (a major determinant of OS) in B[*a*]P-treated rats. As compared with the normal control group, it was obvious that B[*a*]P caused a drastic disturbance in glutathione molecules homeostasis in the positive control group, which was evidenced by a significant ($p \leq 0.05$) reduction in plasma concentrations of GSH and GSSG, as well as the GSH/GSSG ratio, the rates of change were (-47.49, -34.38, and -24.19%), respectively. It is worth mentioning that SMSE somewhat restored these parameters toward normal levels, where treating rats with SMSE at doses of (600 and 800 mg/kg/d) resulted in a significant ($p \leq 0.05$) increase in the levels of GSH by (45.98 and 61.80%); GSSG (28.60 and 39.73%) as well as GSH/GSSG ratios (20.80 and 22.65 %), respectively, compared with those of the positive control group. While SMSE (200 and 400 mg/kg/d) showed no significant differences for the aforementioned parameters except for GSSG for the SMSE (400 mg/kg/d) group. It was clear that SMSE's attenuating effect was concentration-dependent and increased with increasing SMSE dosage.

The current study revealed that exposing rats to B[*a*]P resulted in the generation of OS, as demonstrated by decreased GSH levels and GSH/GSSG ratio. These findings are in accordance with Elhassaneen *et al*. [41], who revealed that treatment of rats with B[*a*]P resulted in a substantial reduction ($p \leq 0.05$) in GSH and GSSG by (-38.35% and -19.30%), respectively as compared to normal rats. Also, Kiruthiga *et al*. [70] and Elhassaneen and El-Badawy [71] found that GSH levels in erythrocytes were reduced significantly ($p \leq 0.05$) after *in vitro* incubation with B[*a*]P. GSH (γ-Glutamyl-Cysteinyl-Glycine), acts as a reducing agent, preventing thiol groups from oxidizing and interacting with harmful ROS members. GSH may serve as a potent ROS scavenger without requiring the assistance of enzymes such as H_2O_2, $^1O_2$, $OH^{\bullet}$, $O_2^{\bullet}$, $ONOO^{-}$, and organic peroxides [16, 72]. The overproduction of ROS decreases antioxidant enzymatic defense system activity and non-enzymatic protein (GSH) levels, compromising the overall antioxidant defense system and impairing its ability to scavenge excess free

radicals, ultimately altering biological system homeostasis [73].

Approximately 90% of the total glutathione concentration in healthy cells and tissues is found in its reduced form (GSH), while the remaining portion exists in its oxidized form (GSSG). The GSH/GSSG ratio is a marker of cellular OS, with a higher GSSG/GSH ratio suggesting severe OS [74]. The drop in GSH and the subsequent increase in GSSG can be considered an indicator of cellular toxicity. Since a reduction in the GSH/GSSG ratio is a common hallmark of many pathological disorders, this ratio is a crucial factor for controlling ROS levels in cells [75]. Moreover, a drop in GSH concentration may suggest a decrease in glutathione reductase activity (GSH-Rd). According to Galkina *et al*. [76], the GSH level may fall owing to decreased GSH-Rd activity, which increases the antioxidant activities of glutathione-*S*-transferase and glutathione peroxidase, both of which employ GSH as a cofactor in their processes. GSH-Rd is necessary for cells to maintain a high GSH/GSSG ratio, as illustrated in reactions (4, 5, and 6).

According to our findings, treating oxidatively-stressed rats with SMSE resulted in partially recovered plasma GSH levels and an improved GSH/GSSG ratio, which may be attributed to the antioxidant properties of SMS. These findings may be corroborated by Aghazadeh *et al*. [46], who observed that *S. marianum* extract improved hepatic GSH content by 65% in steatohepatitis rats induced by a methionine and choline-deficient (MCD) diet compared to extract-untreated MCD-fed rats. Moreover, Anthony and Saleh [77] and Javeed *et al*. [20] clarified that the GC-MS investigation of crude SMS extract revealed seven chemical components responsible for antioxidant activities, including taxifolin, silycristin, silydianin, silybin A, silybin B, iso-silybin A, and iso-silybin B. Additionally, Anthony and Saleh [78] found that these seven main compounds demonstrated remarkable free radical scavenging capacity; notably, taxifolin outperformed the other compounds in terms of radical scavenging activity, as measured by DPPH.

Table 2
Effect of SMSE on glutathione molecules homeostasis in B[*a*]P-treated rats

	Normal control	Control (+Ve)	SMSE (mg/kg/d)-Treated Groups				L.S.D
			200	400	600	800	
Reduced glutathione concentration (GSH, µmol /L)							
Mean	10.04^{a}	5.27^{c}	5.97^{c}	6.12^{c}	7.69^{b}	8.53^{b}	0.89
SD	0.72	0.50	0.07	0.33	0.37	0.69	
% of change	0.00	-47.49	13.22	16.13	45.98	61.80	
Oxidized glutathione concentration (GSSG, µmol /L)							
Mean	0.77^{a}	0.50^{d}	0.57^{cd}	0.59^{c}	0.64^{bc}	0.70^{ab}	0.077
SD	0.03	0.05	0.01	0.04	0.05	0.06	
% of change	0.00	-34.38	14.00	17.40	28.60	39.73	
GSH/GSSG ratio							
Mean	13.13^{a}	9.95^{c}	10.46^{bc}	11.06^{bc}	12.02^{ab}	12.20^{ab}	1.63
SD	0.84	0.97	0.86	1.37	1.21	1.20	
% of change	0.00	-24.19	5.16	11.16	20.80	22.65	

Means in the same row with different superscript letters are significantly different at p ≤ 0.0

3.4. Effect of SMSE on enzymatic antioxidants activities of B[a]P-treated rats

The first line of defense against oxidative damage is endogenous enzymatic antioxidants. The erythrocytes antioxidants enzymes activities in B[*a*]P-treated rats, including glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD), are shown in Table (3). From the obtained data, it was found that injecting rats with B[*a*]P disrupted and suppressed the endogenous enzymatic antioxidant defense system. This was evidenced by a significant ($p \leq 0.05$) drop in GSH-Px, CAT and SOD activities in the positive control group versus the normal one. The rates of change were (-48.82, -34.16, and -47.80%), respectively. Interestingly, treatment of oxidatively stressed rats with SMSE at the concentrations of (200, 400, 600, and 800 mg/kg/d) resulted in a marked improvement in the activities of GSH-Px (13.65, 16.92, 34.25, and 50.25%); CAT (17.71, 18.10, 25.24, and 42.49%) and SOD (2.56, 26.01, 43.22, and 63.37%), respectively in comparison to the positive control group. SMSE (800 mg/kg/d) was the most effective at attenuating oxidative damage to the enzymatic antioxidant defense system, followed by (600 mg/kg/d). Red blood cells (RBCs) may perceived

as carriers of circulating antioxidants that signify exposure to ROS.

RBCs represent a valuable model for investigating OS induced by free radicals (FRs) for different reasons. Firstly, RBCs are frequently subjected to heightened oxidative stress. Secondly, their inability to replace damaged constituents further accentuates their susceptibility to oxidative damage. Thirdly, the partial involvement of polyunsaturated fatty acid side chains in the membrane lipid structure renders them prone to peroxidation. Lastly, RBCs possess enzymatic antioxidant systems [79]. In this context, our findings revealed a significant reduction in erythrocytes' GSH-Px, CAT, and SOD activities in the B[*a*]P-treated group. These findings are consistent with prior research studies [28, 44, 52, 80, 81, 82, 83]. The observed suppression of erythrocyte antioxidant enzymes activities under B[*a*]P administration could be attributed to excessive ROS production during B[*a*]P metabolism, as confirmed by increased levels of blood oxidants (TBARS and NO2); alteration of the redox state, eventually leading to OS and depletion of the antioxidant enzymes.

These results were somewhat compatible with the observations of Kiruthiga *et al*. [84], who observed that incubation of erythrocyte hemolysates with B[*a*]P dramatically reduced antioxidant enzymes activities while raising MDA levels. Antioxidant enzymes constitute the OS defense response system and normalize its damaging effects. According to Banafsheh and Sirous [85], many enzymes work to block the generation of FRs, while others function to scavenge ROS formed directly (primary enzymes) as a result of incomplete reduction of O_2, O_2^{-}, and H_2O_2. The former is scavenged by SOD, whereas the latter is eliminated by CAT and GSH-Px. SOD stimulates the dismutation of $O_2^{\bullet-}$ into H_2O_2 and molecular oxygen, and then the other antioxidant enzymes eliminate H_2O_2 (reaction 1). Additionally, SOD fights NO's superoxide anion. As a result, SOD indirectly lowers the generation of another harmful ROS ($ONOO^{-}$), reaction 2 [86]. CAT catalyzes the breakdown of H_2O_2 into gaseous oxygen and water, as demonstrated in reaction 3 [87]. GSH-Px utilizes H_2O_2 or an organic hydroperoxide as the oxidant and GSH as the electron donor, as shown in reactions 4 and 5 [88]. GSH-Rd employs NADPH as a reducing agent to convert GSSG into GSH, as shown in reaction 6 [72].

(1) $2\,O_2^{\bullet-} + 2H \xrightarrow{SOD} H_2O_2 + O_2$

(2) $O_2^{\bullet-} + NO \xrightarrow{SOD} ONOO^{-}$

(3) $2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$

(4) $2H_2O_2 + 2GSH \xrightarrow{GSH\text{-}Px} 2H_2O + GS{-}SG$

(5) $ROOH + 2GSH \xrightarrow{GSH\text{-}Px} ROH + H_2O + GS{-}SG$

(6) $GSSG + NADPH \xrightarrow{GSH\text{-}Rd} 2GSH + NADP^{+}$

Table 3
Effect of SMSE on enzymatic antioxidants activities of B[*a*]P-treated rats

	Normal control	Control (+Ve)	SMSE (mg/kg/d)-Treated Groups				L.S.D
			200	400	600	800	
Glutathione peroxidase (GSH-Px, U/g Hb)							
Mean	23.33^{a}	11.94^{b}	13.57^{b}	13.96^{b}	16.03^{b}	17.94^{ab}	6.02
SD	2.55	1.59	0.66	1.72	2.69	2.45	
% of change	0.00	-48.82	13.65	16.92	34.25	50.25	
Catalase (CAT, U/g Hb)							
Mean	171.36^{a}	112.82^{c}	132.80^{b}	133.24^{b}	141.30^{b}	160.76^{a}	16.93
SD	15.68	6.60	5.35	10.18	4.73	9.95	
% of change	0.00	-34.16	17.71	18.10	25.24	42.49	
Superoxide dismutase (SOD, U/g Hb)							
Mean	5.23^{a}	2.73^{c}	2.80^{bc}	3.44^{bc}	3.91^{abc}	4.46^{ab}	1.55
SD	1.12	0.30	0.01	1.45	0.86	0.59	
% of change	0.00	-47.80	2.56	26.01	43.22	63.37	

Means in the same row with different superscript letters are significantly different at $p \leq 0.05$

In the present work, SMSE attenuated OS and partially restored the enzymatic antioxidant activities toward normal levels. These effects could potentially attributed to the high content of phenols and flavonoids in SMS. These constituents are particularly essential plant components because of their radical-

scavenging characteristics. In this context, Serçe *et al*. [65] demonstrated that the ethanolic extract from milk thistle seed has powerful antioxidant activities owing to its high contents of total phenolic (620.0±4.93) µg/g and flavonoids (39.32±0.11) µg/g. Furthermore, the hydroxyl groups contained in the extracted molecules of milk thistle extract exhibited a potent FRs scavenging effect. Additionally, the study conducted by Javeed *et al*. [20] demonstrated that the methanolic extracts of different parts of SM showed inhibition of FRs, conferring significant antioxidant activity; the seeds had the greatest DPPH radicals scavenging activity (75.98%), followed by the stem (72.39%), and the leaves (63.21%). There are two possible explanations for silymarin's preventative effect against B[*a*]P in erythrocyte hemolysates, as reported by Kiruthiga *et al*. [84]. The first is that silymarin can serve as a radical scavenger, thus maintaining the activities of antioxidant enzymes; the second is that silymarin prevents/inhibits the synthesis of B[*a*]P metabolites and the formation of additional ROS.

3.5. *Effect of SMSE on antioxidant vitamins concentration of B[a]P-treated rats*

Data demonstrating the effect of SMSE on plasma concentrations of antioxidant vitamins (A, E, and C) in B[*a*]P-treated rats are shown in Table (4). Based on these data, it was evident that B[*a*]P induced a significant ($p \leq 0.05$) decline in plasma concentrations of antioxidant vitamins (A, E, and C) in the positive control compared to the normal control group by (-35.93, -45.78, and -36.80%), respectively. On the other hand, SMSE (200, 400, 600, and 800 mg/kg/day) treatment resulted in a relative improvement in plasma antioxidant vitamins concentration when compared to the positive control group, with the rate of change being (9.35, 20.56, 28.97 and 38.32%) for vitamin A; (14.39, 34.23, 50.32 and 57.43%) for vitamin E; and (10.54, 19.32, 32.67 and 49.71%) for vitamin C, respectively. Most noteworthy, there were no significant variations in antioxidant vitamins levels between the SMSE-treated groups (600 and 800 mg/kg/day) compared to the normal control group.

The lower plasma content of antioxidant vitamins in B[*a*]P-treated groups might be attributed to the overproduction of ROS by B[*a*]P and its metabolites that induce OS, leading to exhaustion/depletion of the antioxidant defense systems, including the antioxidant vitamins. B[*a*]P has been demonstrated to stimulate cytochrome P450 (CYP) enzymes, particularly the CYP1A isoforms [89]. CYP 1A1/1B1 enzymes convert B[*a*]P to epoxide intermediates, which subsequently are metabolized to carcinogenic diol metabolites by CYPs or epoxide hydrolase [90]. Excessive ROS are produced by the CYP-mediated metabolism of B[*a*]P, eventually causing OS [91]. Our results may be supported by previous evidence [80, 92, 93, 94, 95] that has reported that the major contributors to the oxidative effects caused by B[*a*]P are its metabolites and ROS generated during its transformation, which induce OS and reduce the levels of GSH, vitamin C, and vitamin E.

Antioxidant vitamins are essential for OS prevention and control. Vitamin C (ascorbic acid, AA) has a highly electron-donating capacity, enabling it to undergo two-electron oxidations and generate dehydroascorbic acid (DHA), as well as one-electron oxidation, yielding a semi-dehydro-ascorbyl radical. The GSH-dependent mechanism recycles DHA into the active AA form [96]. Superoxide anions, organic peroxides, $^1O_2$, H_2O_2, $OH^{\bullet}$, and HClO are forms of ROS that can be scavenged by AA [16]. Vitamin E is a fat-soluble vitamin that exists in eight distinct forms. Among these forms, α-tocopherol is the most potent and possesses the highest level of antioxidant ability, particularly in cell membranes. It has the capability to donate hydrogen to several types of ROS, such as $^1O_2$, superoxide anions, and peroxyl radicals. The AA then reduces vitamin E's oxidized and radical by-products [97]. Carotenes are the precursors to vitamin A. Carotenoids are potent ROS scavengers with a unique physical and chemical $^1O_2$-suppressing mechanism [98].

The results obtained from the current study have clarified that SMSE treatment restored the antioxidant vitamins level to normal values in a concentration-dependent manner by counteracting B[*a*]P detrimental effects, possibly through the elimination or suppression of ROS generation depending on the high phenols and flavonoid content of the SMS. In this respect, Ligeret *et al*. [99] demonstrated that silibinin has powerful antioxidant characteristics since it lowers the release of $O_2^{\bullet -}$, diminishes the accumulation of LPO products, eliminates FRs, restores GSH levels, and improves GSH-related enzyme activities. The presence of OH on the third, fifth, and seventh carbon atoms of the silibinin, silydianin, and silychristin components may explain their higher antioxidant activity and the elimination of ROS generated during B[*a*]P metabolism [70]. Besides that, Akbari-Kordkheyli *et al*. [100] indicated that the potent FRs scavenger with anti-inflammatory activities of *S. marianum* extract is silymarin, which accounts for 70-80%, with silibinin as the main ingredient, can protect tissues from OS through various processes such as FRs scavenging, reducing inflammatory cytokines, suppressing cellular death, and improving antioxidant enzymes expression.

Table 4
Effect of SMSE on antioxidant vitamins concentration of B[*a*]P-treated rats

	Normal control	Control (+Ve)	SMSE (mg/kg/d)-Treated Groups				L.S.D
			200	400	600	800	
Vitamin A (Retinol, μmol/L)							
Mean	1.67^{a}	1.07^{c}	1.17^{bc}	1.29^{abc}	1.38^{abc}	1.48^{ab}	0.467
SD	0.19	0.15	0.09	0.28	0.56	0.30	
% of change	0.00	-35.93	9.35	20.56	28.97	38.32	
Vitamin E (Tocopherol, μmol/L)							
Mean	31.41^{a}	17.03^{c}	19.48^{bc}	22.86^{bc}	25.60^{ab}	26.81^{ab}	6.93
SD	2.83	2.88	1.31	3.32	4.19	6.68	
% of change	0.00	-45.78	14.39	34.23	50.32	57.43	
Vitamin C (Ascorbic acid, μmol/L)							
Mean	74.29^{a}	46.95^{c}	51.90^{bc}	56.02^{bc}	62.29^{ab}	70.29^{a}	12.97
SD	6.56	6.64	1.14	7.61	6.34	11.51	
% of change	0.00	-36.80	10.54	19.32	32.67	49.71	

Means in the same row with different superscript letters are significantly different at $p \leq 0.05$

3.6. Effect of SMSE on liver apoptosis biomarkers in B[a]P-treated rats

The generation of ROS via B[*a*]P and the subsequent OS play a decisive role in cell apoptosis. A flow cytometry analyzer was used to assess the effect of SMSE on the expression of apoptosis-related proteins in the hepatic tissues of B[*a*]P-treated rats. As shown in Table (5), compared to the normal control group, intraperitoneal injection of B[*a*]P at a dose of (125 mg/kg/b. wt.) in the positive control group induced a marked increase ($p \leq 0.05$) in caspase-3 expression (a cell apoptosis executor) and Bax protein expression (a pro-apoptotic protein that mediates cell death); in contrast, Bcl-2 (an anti-apoptosis marker) protein expression was significantly diminished by a rate of change (67.58, 91.55 and -65.80%), respectively. On the other side, SMSE treatment attenuated these changes significantly ($p \leq 0.05$) in a dose-dependent manner by either upregulating the protein expression of Bcl-2 (39.49, 43.65, 73.03 and 105.69%) or downregulating the expressions of caspase-3 (-18.39, -27.19, -29.93 and -34.80%) and Bax (-19.39, -29.82, -32.71 and -41.02%) effectively in SMSE treated groups (200, 400, 600 and 800 mg/kg/d), respectively when compared to the positive control group.

Our data confirmed that B[*a*]P activates apoptosis by altering the expression of proteins related to apoptosis. These findings align with those of Khattab *et al*. [101], who noticed a highly marked elevation in Bax and caspase-3 and a highly significant decrease in Bcl-2 in kidney tissues of rats that received 50 mg/kg body weight of B[*a*]P twice a week for 28 days. B[*a*]P disrupts the equilibrium between reactive intermediate generation and biological ROS detoxification. B[*a*]P in the cellular environment causes a variety of molecular changes, including lipid peroxidation and protein oxidation, leading to OS [92, 102].

OS triggers multiple apoptotic signaling pathways due to increased ROS production or reduced antioxidant enzymes activities. ROS is closely linked to the activation of cell apoptosis through changing the redox state of the cell and creating an imbalance in the protective mechanism that results in damage to cellular molecules like lipids, proteins, and DNA, ultimately leading to cell death via necrotic and apoptotic processes [1, 103, 104, 105]. Furthermore, ROS plays a crucial role in triggering apoptosis by promoting caspase activation. Caspases, specifically caspase-3, are key apoptotic effectors that cause cytoskeletal degradation, nuclear demise, and other apoptotic cellular alterations [106]. Lin *et al*. [93] found that a 250 mg/kg intraperitoneal injection of B[*a*]P into the embryo after a week caused neural tube defects in mice. These scientists demonstrated that B[*a*]P exposure markedly elevated the expression of OS genes such as superoxide dismutase (SOD1&2*)* and cytochrome p450 (CYP1A1) while reducing glutathione peroxidase 1 (GPX1). The neural epithelium of B[*a*]P-treated mice embryos showed increased apoptosis and raised caspase-3 expression. Likewise, Kim *et al*. [107] observed that B[*a*]P treatment of JEG-3 and BeWo human choriocarcinoma cancer cells resulted in elevated levels of Bax protein expression, reduced Bcl-xl protein expression, and increased the proportion of apoptotic cells. These data imply that B[*a*]P may induce apoptosis by increasing ROS levels while also activating the endoplasmic reticulum stress response.

The results of the current study indicated that SMSE attenuates B[*a*]P-induced hepatic apoptosis by inhibiting the apoptotic proteins caspase-3 and Bax and activating the anti-apoptotic mediator Bcl-2. SMS has flavonoid and phenolic components, including silymarin and silibinin. These components possess the potential to inhibit ROS formation, which may explain the extract's anti-hepatic apoptotic action.

Table 5
Effect of SMSE on liver apoptosis biomarkers in B[*a*]P-treated rats

	Normal control	Control (+Ve)	SMSE (mg/kg/d)-Treated Groups				L.S.D
			200	400	600	800	
Caspase-3 (%), (a cell apoptosis executor)							
Mean	32.16^{e}	53.89^{a}	43.98^{b}	39.24^{c}	37.76^{c}	35.14^{d}	2.078
SD	0.24	0.86	0.19	1.43	1.53	1.72	
% of change	0.00	67.58	-18.39	-27.19	-29.93	-34.80	
Bax (%), (a pro-apoptosis marker)							
Mean	30.69^{e}	58.79^{a}	47.39^{b}	41.26^{c}	39.56^{c}	34.67^{d}	3.157
SD	1.34	2.41	1.49	2.02	1.87	1.24	
% of change	0.00	91.55	-19.39	-29.82	-32.71	-41.02	
Bcl-2 (%), (an anti-apoptotic marker)							
Mean	53.47^{a}	18.29^{e}	25.51^{d}	26.27^{d}	31.65^{c}	37.62^{b}	2.827
SD	1.55	1.63	0.83	1.43	1.84	1.99	
% of change	0.00	-65.80	39.49	43.65	73.03	105.69	

Means in the same row with different superscript letters are significantly different at $p \leq 0.05$

According to Kren and Walterová [108], the hepatoprotective effects of *S. marianum* extract against prooxidants are believed to be primarily mediated by free radical scavenging. The extract enhances GSH levels in the liver, which serve as the first line of defense against ROS. Kwon *et al*. [109] discovered that silymarin enhances hepatic glutathione production by increasing the availability of cysteine, which in turn may improve the liver's antioxidant capacity. In addition, Federico *et al*. [110] reported that silymarin, a milk thistle extract, contains the bioactive component silybin, which has potent biological activity. It has proven to be effective in treating a variety of liver diseases, including hepatocellular carcinoma, chronic liver diseases, and cirrhosis, due to its remarkable antioxidant, anti-inflammatory, and antifibrotic properties.

There is a body of evidence indicating that *S. marianum* protects against cell apoptosis induced by diverse models in experimental animals. A study conducted by Aghazadeh *et al*. [46] concluded that *S. marianum* extract effectively inhibited the activation of procaspase-3 to caspase-3 in nonalcoholic steatohepatitis-affected rats. Prabu and Muthumani [64] mentioned that silibinin, a bioflavonoid in *S. marianum*, suppressed arsenic-induced apoptosis and renal tubular destruction by inhibiting caspase-3 expression, suggesting that silibinin's anti-apoptotic effects might be related to its anti-inflammatory and FRs scavenging properties. In addition, Kim *et al*. [111] found that silymarin dramatically reduced acute liver damage caused by restraint stress by inhibiting N-terminal kinase (known to induce apoptosis) activation and subsequent apoptotic signaling. Finally, a recent study by Yassin *et al*. [112] discovered that total extract of *S. marianum*, silibinin, and silymarin resulted in the downregulation of apoptotic proteins p53 and caspase-3 and the upregulation of anti-apoptotic mediator Bcl-2 in rats treated with diethylnitrosamine, 2-acetylaminofluorene, and CCl_4.

Conclusion

Oxidative stress is an imbalance between the generation of free radicals and the body's capability to neutralize their damaging effects. This imbalance ultimately leads to the initiation and advancement of various chronic and degenerative illnesses. Natural antioxidants are essential compounds for counteracting oxidative chain reactions and protecting the body from the damaging effects of toxic free radicals that trigger oxidative stress. The current study indicated that the ethanolic extract of the wild *S. marianum* seed has potent antioxidant activities, which effectively attenuated the oxidative stress and liver apoptosis induced by benzo[*a*]pyrene (B[*a*]P). The attenuating effect might be attributable to the seeds' high content of several bioactive compounds, such as silymarin, silybin, and taxifolin, which eliminate ROS generated by B[*a*]P and its metabolites. Based on these findings, wild *S. marianum* seeds could offer new opportunities for the developing value-added products for human nutrition and/or dietary

supplements to prevent or treat chronic and degenerative disorders induced by oxidative stress.

Conflict of interest

The authors declare that they have no conflict of interest in publishing this paper.

Abbreviations

B[*a*]P: benzo[*a*]pyrene; BD: basal diet; CAT: catalase; FRs: free radicals; GSH: reduced glutathione; GSH-Px: glutathione peroxidase; GSH-Rd: glutathione reductase; GSSG: oxidized glutathione; LPO: lipid peroxidation; MDA: malondialdehyde; NADPH: nicotinamide adenine dinucleotide phosphate; OS: oxidative stress; RNS: reactive nitrogen species; ROS: reactive oxygen species; SM: *Silybum marianum*; SMS: *Silybum marianum* seeds; SMSE: *Silybum marianum* seed ethanolic extract; SOD: superoxide dismutase; TBARS: thiobarbituric acid reactive substances.

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