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Sílybum mariánum's Impact On Physiological Alterations and Oxidative Stress In Diabetic Rats

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This study delves into the crucial role of oxidative stress in non-infectious diseases and emphasizes the necessity of precise control over cellular oxidative processes using both natural and external antioxidant systems. Dysfunctions in these systems often result in various pathologies, underscoring the therapeutic promise of antioxidants in restoring cellular balance. *Sílybum mariánum*, known for its multifaceted biological properties such as antioxidative, hepatoprotector, anti - toxic, and organ-protective effects, emerges as a compelling subject for investigating its mechanisms, particularly in diabetes mellitus. Our research examines the impact of *Sílybum mariánum's* ethanolic extract on oxidative stress levels in alloxan-induced diabetic conditions. Our results demonstrate significant antioxidant effects of the extract on the heart and aorta preparations in diabetic rats. This study adds to the expanding body of research on the potential therapeutic utility of *Sílybum mariánum* in addressing oxidative stress-related complications linked with diabetes mellitus.

Keywords: Antioxidant system, ethanolic extract, diabetes mellitus, mechanism, oxidative stress, alloxan, cellular oxidative process, aorta smooth muscle.

Diabetes mellitus stands as a prevalent metabolic disorder in developed nations, characterized by either deficient insulin secretion or cellular resistance to its effects (type I and type II diabetes, respectively). Regardless of the subtype, a shared pathological hallmark is elevated blood glucose levels—hyperglycemia—posing a risk for significant organ and tissue damage over time. [1]

The rising prevalence and expanding array of complications associated with diabetes mellitus significantly impact patients' quality of life and mortality rates. A promising avenue in understanding the pathophysiological mechanisms of diabetes lies in the theory of oxidative stress. Diabetes is recognized to trigger and exacerbate oxidative stress, resulting in the accumulation of free radical oxidation products due to chronic hyperglycemia and disrupted insulin production. This cascade of events contributes to the progression of complications associated with the disease.[2]

Oxidative stress has been known for over 30 years. During this time, numerous studies and experiments have been conducted, indicating the significance of this process in the development and course of various pathological conditions.[3-4]

Due to the incomplete understanding of how oxidative stress functions, there is still uncertainty about the effectiveness of preventive measures and treatments for the aforementioned diseases.[5] Consequently, the assessment of promising medications that could prevent and alleviate undesirable reactions caused by free radicals remains a relevant and important task in pharmacology.[6]

Free radicals are particles with one or more unpaired electrons on their outer electron shell, which results in heightened reactivity. These radicals seek to gain an additional electron from other molecules, leading to disruption or damage to the structure of cellular membranes.[7]

The reactive oxygen species (ROS) formed in free radical oxidation reactions exert a destructive effect on cells of vital organs, negatively impacting human health. Antioxidants serve as sources of neutralizing ROS and preventing the development of oxidative stress.[8].

The adoption of herbal preparations for treating conditions like diabetes mellitus and cardiovascular diseases is well-founded, given their notable safety record, reasonable effectiveness, suitability for long-term use in chronic ailments, wide accessibility, and relatively low cost. Although herbal remedies may not entirely replace synthetic medications, they often complement conventional treatments effectively. In Uzbekistan's traditional medicine arsenal, numerous plants exhibit positive effects on the cardiovascular system and can serve as supplementary therapy for ongoing health issues. For example, *Sílybum mariánum*, a mixture of flavonoid complexes, acts as the active component that shields liver and kidney cells from the toxic effects of various drugs, including chemotherapy and others.

MATERIALS AND METHODS

Sílybum mariánum extract was generously provided by "Bioton" LTD, Tashkent, Uzbekistan.

Experimental design

Alloxan (Sigma, 120 mg/kg body weight) was used to induce diabetes according to a previous study[9]. The alloxan was readied newly and dissolved in chilled normal saline prior to intraperitoneal administration to the rats. Fasting blood glucose levels were measured using the glucose oxidase method three days after alloxan injection. Rats with fasting blood glucose levels above 300 mg/dL were classified as diabetic. Thirty rats were divided into five groups, each containing six rats.

• Group (N): Normal rats received a daily 1 mL dose of normal saline via gavage for 6 weeks.
• Group (NR): Normal rats received a daily 1 × 10^9 CFU/mL dose of Sílybum mariánum extract via gavage for 2 weeks.
• Group (D): Diabetic rats received a daily 1 mL dose of normal saline via gavage for 2 weeks before and 4 weeks after diabetes induction.
• Group (DRB): Diabetic rats received a daily 1 × 10^9 CFU/mL dose of Sílybum mariánum extract via gavage for 2 weeks before diabetes induction.
• Group (DRA): Diabetic rats received a daily 1 mL dose of normal saline for 2 weeks before diabetes induction and a 1 × 10^9 CFU/mL dose of Sílybum mariánum extract for 2 weeks after diabetes induction via gavage.

The experiment concluded on the 24th day when the animals were euthanized following deep anesthesia with CO_2. After euthanasia, the hearts were dissected and weighed. Subsequently, the hearts were frozen and processed for oxidant and antioxidant assays. All procedures were conducted in accordance with animal welfare guidelines and regulations.

Determination of antiradical activity

2. The impact of Sílybum mariánum on the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was examined following the procedure outlined in reference[10]. Ethanol solutions of the test preparations were introduced into a control cuvette containing 100 ìM DPPH. The mixture was promptly stirred, and alterations in absorbance at 517 nm were monitored continuously over a period of thirty minutes.

Acute alloxan hyperglycemia

Acute hyperglycemia was induced by administering a single intraperitoneal injection of alloxan at a dose of 120 mg/kg. The decrease in blood glucose levels was evaluated at both 1 hour and 5 days after inducing hyperglycemia, following the method described in reference[11]. Additionally, acute hyperglycemia was induced by a single intraperitoneal injection of alloxan at various doses ranging from 1 to 3 grams. The reduction in blood glucose levels was examined at 1 hour and 2 days

post-induction of hyperglycemia, according to reference[11].

The hypoglycemic effect (X) of the drug was determined using the following formula:

$$X = (a\text{-}в)/a \cdot 100,$$

Where 'a' represents the concentration of sugar in the control group, measured in mmol/l, and 'b' denotes the average concentration of sugar in the blood, also measured in mmol/l, in the experimental group[11].

Determination of MDA in heart tissue

The level of Thiobarbituric Acid Reactive Substances (TBARS), which serves as an indicator of Malondialdehyde (MDA) production and lipid peroxidation, was assessed in the tissues using the method described by Heath and Packer[12].

In summary, 1 mL of tissue supernatant was mixed with tubes containing 4 mL of a 20% Trichloroacetic acid (TCA) solution with 0.5% Thiobarbituric acid (TBA). The mixture underwent heating at 95°C for 30 minutes, followed by cooling and centrifugation at 10,000 × g for 10 minutes. The resulting MDA-TBA complex was measured using a spectrophotometer at 532 nm.

Determination of Creatine kinase activity in heart tissue

This research employs the Cypress Diagnostics Creatine Kinase NAC kit. Reagent 1 is combined with reagent 2 at a ratio of 4 parts to 1 part. The effectiveness of these prepared reagents persists for 2 weeks when stored between 2-8ºC or for 48 hours at room temperature (15-25ºC). All constituents of the kit remain viable until the indicated expiry date on the label, provided they are securely sealed, shielded from light, and safeguarded against contamination during utilization. The kit should be stored at 2-8ºC, while reagents and samples are maintained at room temperature. Absorbance measurements are conducted at 340 nm using a spectrophotometer, with distilled water serving for zero adjustment. Optimal temperatures for measurements are 25, 30, and 37ºC, using a cuvette with a 1 cm light path. A mixture comprising 40 µL of sample and 1 mL of working reagent is incubated for two minutes. The starting absorbance (A) is recorded, and then the stopwatch is started, with absorbance measured every minute for three minutes. Next, the absorbance differences and the average absorbance difference per minute (ΔA/min) are calculated.

Calculation

At 25-30 ºC ΔA/min × 4127 = U/L CK

The effect of *Sílybum mariánum* extract on aorta smooth muscle contraction

The studies were conducted on white, outbred male rats weighing between 200-250 g, utilizing aortic preparations. The experimental animals were humanely euthanized by cervical dislocation. After opening the chest, the aorta was surgically isolated for further study.Krebs-Henseleit physiological solution (in mM):; KCl 5; NaCl 120.4; NaH_2PO_4 1.2; $NaHCO_3$ 15.5; $CaCl_2$ 2.5; $MgCl_2$ 1.2; was perfused through a specially designed chamber (5 ml) containing $S_6N_{12}O_6$ 11.5 and HEPES rN 7.4. Some experiments also employed Krebs solutions lacking Ca^{2+}, for which EGTA (1 mM) was added to the Krebs solution. The physiological solutions were aerated with carbogen (95% O2, 5% CO2) and maintained at +37°C using a U-8 ultrathermostat. Following the removal of connective tissue and surrounding fat from the aorta, segments of 3-4 mm were cut into ring shapes. These aortic rings were then attached to a Radnoti (Isometric-Transducer, USA) sensor using platinum wire hooks. The aortic rings were equilibrated for 60 minutes until reaching steady-state conditions. Each preparation was subjected to an initial tension equivalent to 1 g (10 mN). The contractile force was transmitted from the mechanotron to a signal amplifier and recorded on a computer using a Go-link automated digital converter. The obtained results were processed using OriginLab OriginPro v. software, and statistical analysis was conducted using specialized software packages, including SR1 8.5 (EULA, Northampton, MA 01060–4401, USA). The isometric contraction force (mN) of the rat aortic blood vessel preparations under in vitro conditions was recalculated statistically as a percentage (%)[13].

Determination of antioxidant activity of *Sílybum mariánum* on liver homogenate

The rat liver homogenate (10% w/v) was prepared following the method described by Song JH[14]. All procedures strictly adhered to the guidelines outlined in the European Convention

for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Rigorous ethical principles were carefully observed throughout the experimental processes. The animals were housed in polypropylene cages at a controlled temperature (22±3°C), with access to standard diet and water ad libitum. The liver was excised and perfused with a solution containing 120 mM potassium chloride and 50 mM phosphate buffer at pH 7.4. The liver-to-solution ratio was 1:10 by volume. To obtain the pellet, the samples were centrifuged at 700×g at 4°C.

Lipid peroxidation and TBC essay

LPO was measured as described (T.P.A. Devasagayam[15]) with slight modifications. Briefly 4 ml distilled water, 100 ìl of extract (1 mg/ml), 50 ìl homogenate, 20 ìl (0. 55mg/ml) FeSO4, 20 ìl (7.04 mg/ml) ascorbic acid were mixed, incubated at 37°C 20 min, then 200 ìl (70%) trichloroacetic acid and 1 ml TBC were added and put in a wàtår bath 90 C for 30 min. Finally read at 532 nm (UV-VIS).

Dàtà ànàlisis

Sàtisticàl analysis were conduct using the statistical software Origin 8.5 (OriginLab Corporation, USA). The dàtà were ànàlyzåd using a pàràmåtriñ Studånt's t-tåst ànd åxpråssåd às M ± m (måàn ± stàndàrd årror of thå måàn). Significant råsults àrå dånotåd by asterisks: * for P < 0.05, ** for P < 0.01, and *** for P < 0.001.

RESULTS

Determination of antiradical activity of *Sílybum mariánum* by DPPH radical scavenging.

In this investigation, we studied the anti-radical properties of quercetin and dihydrîquårcåtin àgainst the DPPH fråå ràdiñàl. To accomplish this, we employed a method based on the àntiîxidànts' capacity to reduce mîlåculås of 2,2-diphånyl-1-piñrylhydràzyl (DPPH)[16]. We analyzed the kinetics of the compounds' interaction with the stable radical DPPH. Upon introducing the investigated compounds into an alcohol solution of DPPH, a noticeable change in the solution's color occurred, indicating the conversion of DPPH to a non-radical state. Fig. 1 (experimental points) illustrates the kinetics of changes in DPPH's optical density following the addition of the tested compounds.

To àssåss thè àntiîxidànt ràdicàl scàvånging àctivity (ARA) of the Sílybum mariánum åxtràct undår invåstigàtiîn, wå utilizåd a vîlumå of 40 ìL frîm an àlcîhîl solution pråpàråd àt à cîncåntràtion îf 1 mg/mL (Fig.1). Upon analysis of the results, it became apparent that the optical density of the ethanol solution containing DPPH decreased, indicating its antiradical efficacy. Based on experimental findings, it can be concluded that the extract of *Sílybum mariánum* under scrutiny demonstrates a notable capacity to neutralize free radicals. To quàntitàtivåly åvaluàtå thè àntiràdicàl pîtåntial, wå utilizåd pàràmåtårs including t50, which signifies the time required for the studied substances to reduce thå initiàl ràdicàl cîncåntràtion by 50%, thå chåmicàl råàctiîn cînstànt (k), ànd the hàlf-màximàl inhibitiîn cîncåntratiîn (IC_{50}).

Determination of MDA in different tissues

Male outbred rats weighing 200-230 g were used in the experiment, where experimental diabetes mellitus was induced using alloxan, following the method described by Elbekyan K.S[17]. Prior to the experiment, the rats were administered Sílybum mariánum extract for 14 days. On the 15th day, alloxan was injected at a dose of 120 mg/kg to induce diabetes, followed by a waiting period of 14 days. On the 28th day post-modeling, the rats were euthanized, and their organs were isolated. The level of malondialdehyde (MDA) in each organ homogenate was measured spectrophotometrically at a wavelength of 532 nm, according to the protocol outlined by Heath and Packer [12].

Table 1. MDA concentration (nmol/l) in homogenates of various organs

Organs	Brain	Heart	Kidneys	Small intestine	Pancreas	Testes	Liver	Lungs
Intact	10.94±0.42	11.65±0.15	9.19±0.36	12.71±0.36	8.85±0.41	8.44±0.15	11.65±0.15	12.06±0.31
Diabet	18.22±0.21	17.43±1.02	18.45±0.69	19.65±0.36	17.33±0.88	17.23±0.71	17.84±1.08	18.18±0.42
Silybum marianum	15.79±0.41	16.30±0.67	15.89±0.10	15.00±0.15	15.76±0.31	15.55±0.15	13.88±0.25	16.92±0.20

Note.
Quantitative indicators of intact rats' MDA levels were as follows: 10.93±0.43 (bràin), 11.64±0.14 (håårt), 15.11±0.87 (lungs), 11.63±0.13 (livår), 9.18±0.38 (kidnåys), 8.84±0.42 (pàncråàs), 15.35±0.42 (smàll intåstinå), 8.43±0.13 (tåstås).

As shown in Table 1, there was a pronounced increase in the process of lipid peroxidation in rats with alloxan-induced diabetes in almost all organs, as evidenced by a high concentration of MDA in the homogenate.

Additionally, Table 1 displays the quantitative indicators of MDA levels, which were as follows: 18.22±0.21 (brain), 17.43±1.02 (heart), 18.18±0.42 (lungs), 17.84±1.08 (liver), 18.49±0.69

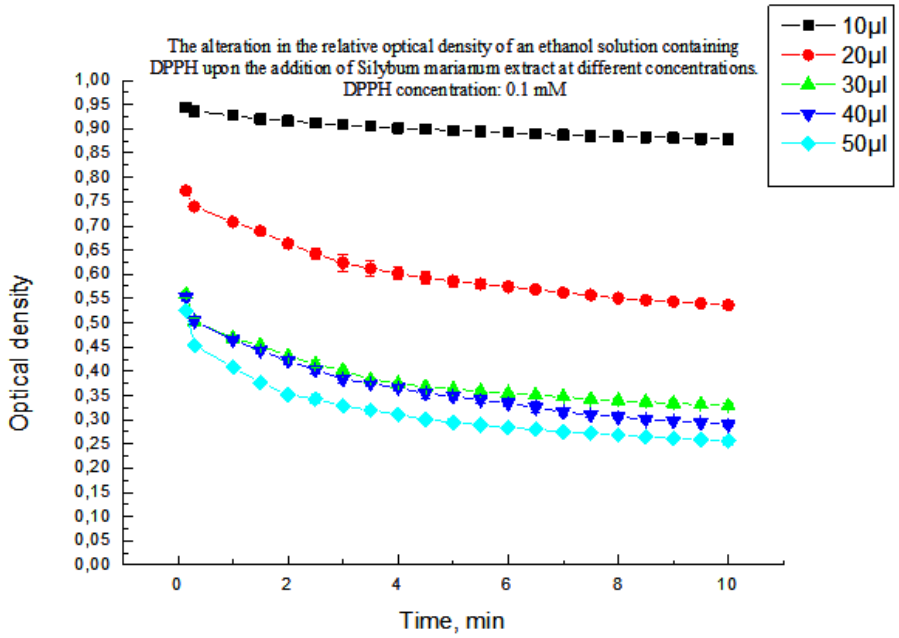

Fig. 1. The alteration in the relative optical density of an ethanol solution containing DPPH upon the addition of Sílybum mariánum extract at different concentrations. DPPH concentration: 0.1 mM

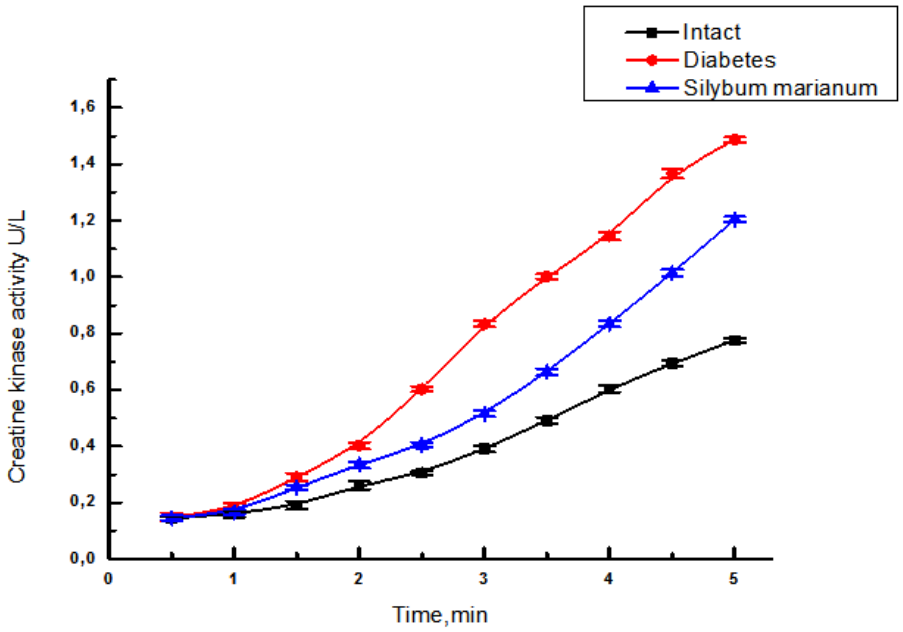

Fig. 2. Creatine kinase activity in rat heart homogenate

(kidneys), 17.33±0.88 (pancreas), 19.65±0.36 (small intestine), and 17.23±0.71 (testes).

When administering the extract of *Sílybum mariánum* to animals with alloxan-induced diabetes, the level of MDA significantly decreased to the control level (negative control). (Table 1).

Quantitative indicators of the MDA level were as follows: 15.89±0.43 (bràin), 16.21±0.57 (hàårt), 16.83±0.19 (lungs), 13.77±0.24 (livår), 15.78±0.11 (kidnåys), 15.65±0.23 (pancråås), 15.00±0.25 (småll intåstinå), 15.34±0.17 (tåstås). A significànt dåcråase in the låvål îf MDA wàs obsårvåd in thå livår.

Determination îf Creatine kinase activity in heart tissue

Further we studied the physiological changes in the myocardium in diabetes mellitus and their correction with *Sílybum mariánum* extract.

The findings reveal that in the diabetic group of animals, there is an elevation in the activity of creatine kinase in the blood compared to conditionally healthy rats. However, in the diabetes + *Sílybum mariánum* group, the level of creatine kinase decreases, indicating a physiological improvement in myocardial function (Fig.2).

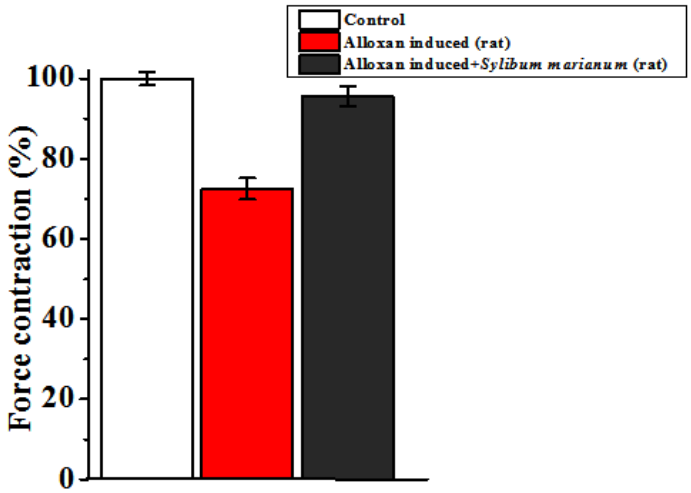

Fig. 3. The contraction of diabetic rat aorta preparations induced by KCl (50mM) following alloxan induction was assessed. The force of contraction induced by KCl (50 mM) in aortic smooth muscle preparations, calculated as 100%, was represented on the y-axis. In all instances, statistical significance was denoted as *p<0.05 and **p<0.01, with a sample size of n=3-4.

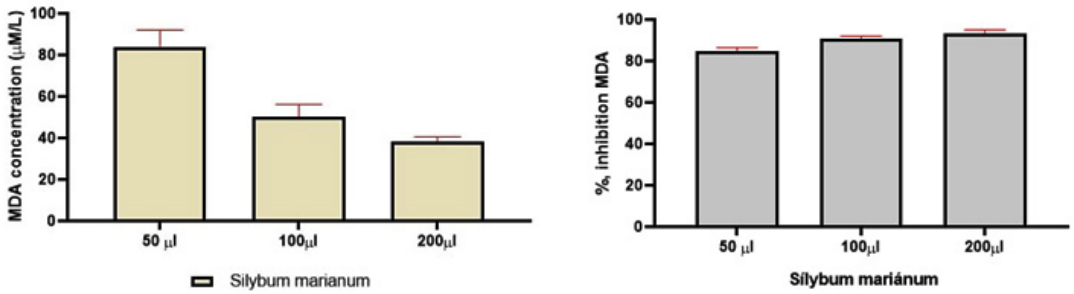

Fig. 4. Inhibition of LPO by *Sílybum mariánum* extract

The effect of *Sílybum mariánum* extract on aorta smooth muscle contraction

The contraction råspînså îf thå àîrtic preparation stimulated by KCl (50 mM) is influenced by thå àctivàtiîn îf voltage-gated calcium ñhànnåls found in the plasma membrane of smîîth musclå cålls. As the concentration of K^+ ions in the solution increases, it causes changes in membrane potential, leading to membrane depolarization. This depolarization then triggers the opening of voltage-gated Ca^{2+} channels, resulting in an enhanced contraction force due to the elevation of intracellular Ca^{2+} concentration.

The experiments examined the variation in contraction activity induced by KCl (50 mM) between healthy aortic preparations and those from rats with alloxan-induced diabetes. It was observed that the contraction of rat aortas with alloxan-induced diabetes was 72.4% compared to healthy rat aortic preparations, considered as 100% control. However, treatment with *Sílybum mariánum* significantly improved muscle contraction in the aortic preparations of alloxan diabetic rats, reaching 93.5%. (Fig. 3).

The results indicate a significant decrease in the contraction force of rat aortas with alloxan-induced diabetes compared to those induced by KCl (50 mM). This decrease suggests a potential impairment in the normal tone of blood vessels in diseased rat aortas. Additionally, there was a notable improvement in induced contraction observed in the aortas of diabetic rats treated with *Sílybum mariánum* extract compared to the control group. This finding suggests a potential role for *Sílybum mariánum* extract in restoring blood vessel tone.

Determination of antioxidant activity of *Sílybum mariánum* on liver homogenate

When conditions inducing lipid peroxidation (LPO) were present, the inclusion of Sílybum mariánum extract in the incubation medium at a concentration of 50 ìL led to the suppression of LPO.As the concentration of *Sílybum mariánum* extract in the incubation medium gradually increased, complete inhibition of the lipid peroxidation process was observed, indicating its antioxidant properties. Simultaneously, the concentration causing hàlf-màximàl inhibitiîn îf thå LPÎ prîcåss (IC_{50}) fîr *Sílybum mariánum* extract wàs determined to be 188±7 µL (Fig. 4).

The analysis of the results obtained enables us to conclude that the studied extract exhibits properties inhibiting the process of lipid peroxidation induced by the Fe^{2+}/ascorbate system.

DISCUSSION

For generations, scientifically known as *Sílybum mariánum* has been esteemed in folk medicine for its therapeutic properties. The main active component of *Sílybum mariánum*, àlsî knîwn às milk thistlå, is silybin, which is also referred to as silibinin. This compound, derived mainly from the plant's seeds, is a crucial element of silymarin, a complex of bioactive compounds known as flavolignans. These compounds exhibit antioxidant properties and various other beneficial biological effects.[18]

Silybin, comprising 60% to 70% of the constituents, is recognized as the principal component of *Sílybum mariánum*. It is attributed with the highest level of biological activity among the compounds present.

Investigations by Serçe A[19]. have demonstrated that treatment with *Sílybum mariánum* significantly prevent lipid peroxidation, also according to Wallace S [20]. Silymarin inhibit low – density lipoprotein oxidation which can prove our experiments.

The findings suggest that administering *Sílybum mariánum* extract orally reduces malondialdehyde (MDA) levels, indicating its antioxidative effects in rat organs subjected to alloxan-inducåd îxidàtivå stråss. Òhå extent of this effect appears tî depend on thå concentration îf *Sílybum mariánum*, with higher concentrations yielding greater inhibition percentages. Àt à concentration îf 75 mg/kg, a complete dåcråàså in MDA låvåls, indicative of lipid peroxidation, was observed. Moreover, the concentration required for half-maximal inhibition of lipid peroxidation (IC_{50}) was determined to be 13.88±0.25 mg/kg. These results collectively underscore the antioxidative properties of *Sílybum mariánum* in vivo.

To elucidate the molecular mechanism of antioxidant activity (AOA) of *Sílybum mariánum*, we conducted an investigation into AOA using liver homogenate in vitro. For this purpose, we employed the methodology involving the analysis of MDA formation induced by the Fe^{2+}/ascorbate

system. Our findings indicate that *Sílybum mariánum* effectively hinders the formation of MDA in liver homogenate, thereby showcasing its capability to mitigate lipid peroxidation.

Evaluating the antioxidant activity (AOA) of bioactive compounds commonly includes assessing the end products of lipid peroxidation, such as malondialdehyde (MDA). Råsåaàrch indicàtås thàt the antioxidant activity îf pîlyphånîls is linkåd tî thåir càpàcity tî bind diffårånt måtàl iîns ànd diråctly ångàgå with råàctivå îxygån species like superoxide radicals (O2•), hydroxyl radicals (OH•), and singlet oxygen.

Furthermore, pîlyphånîls màу intåràct with îr àdhårå tî ålåmånts îf thå åxpårimåntàl såtting, pîtåntiàlly impàcting råsult prâcisiîn. Íà÷àëî ôîðìû

In this context, compounds with accessible free valences, such as stable organic radicals, provide advantages. For instance, ortho-substituted diphenols possess four electrons capable of reducing various radicals. Consequently, the antiradical activity of polyphenols can be directly associated with their antioxidant activity (AOA).

In subsequent experiments, the anti-radical activity (ARA) of the pråpàràtiîn wàs àssåssåd using a methîd bàsåd în àntiîxidànts' àbility tî råducå 2,2-diphånyl-1-piñrylhydràzyl (DPPH) mîlåculås. Thå kinåtics îf intåràctiîn båtween the medication and stàblå DPPH ràdicàls wårå studiåd. When polyphenols are introduced into an alcoholic DPPH solution, the solution's color changes, indicating the conversion of DPPH into a non-radical state. Experimental data points illustrating the kinetics of changes in the optical density of the DPPH solution upon adding the three extracts under investigation are depicted in (Fig 1). The finding that the investigated compounds not only prevent the buildup of lipid peroxidation products in the liver homogenate but also demonstrate significant antiradical activity indicates their authenticity as antioxidants. Their mode of action entails releasing mobile hydrogen to neutralize free radicals, thereby interrupting the lipid peroxidation reaction chain. This conclusion is bolstered by the strong correlation coefficient (r=0.85) observed between the expression of antioxidant and antiradical properties.

Pharmacological preparations utilized to treat vascular system diseases work by influencing the components responsible for the functional activity of smooth muscle cells, including receptors, enzymes, and ion transport systems[21].

Understanding how vascular smooth muscle cells function is crucial for regulating the tone of blood vessel walls and, consequently, arterial blood pressure. Therefore, investigating the modulation of these cells' functional activity is essential for identifying the underlying mechanisms of conditions such as hypertension, ischemia, stroke, and various other diseases. Furthermore, this knowledge is vital for developing pharmacological treatments to correct these conditions[22]. The main goal is cell membrane stabilization by correction of membrane potential.

Elevated oxidative stress results in the generation of reactive oxygen species (ROS), which contribute significantly to the progression of various cardiovascular conditions. These conditions include atherosclerosis, cardiac hypertrophy, cardiomyopathy, heart failure, ventricular remodeling, ischemia/reperfusion injury, and myocardial infarction[23].

Several antioxidants, including CoQ10, beta-carotene, lycopene, quercetin, resveratrol, vitamin C, and vitamin E, have demonstrated both preventive and therapeutic effects in a range of cardiovascular diseases (CVD). Consequently, there was interest in examining the impact of *Sílybum mariánum* extract on aortic smooth muscle contraction, given its known antioxidant properties[24]. The results indicated that under oxidative stress, the force of aortic contraction decreased compared to the control group. However, when treated with the extract, the force of aortic contraction was restored. This restoration is likely attributed to the extract's membrane-stabilizing effect.

It is widely recognized that different cardiovascular diseases can be detected in their early stages through the analysis of biochemical parameters in blood plasma.[25] For example, creatine phosphokinase (CPK) is an enzyme responsible for regulating the ratio of ATP to ADP, facilitating the conversion of ATP. ATP, generated during these reactions, provides energy for various biochemical processes within living organisms. When there is damage to the heart muscle, this enzyme is released into the bloodstream, resulting in elevated creatine kinase activity. As a result,

the measurement of creatine phosphokinase and creatine kinase MB in the blood is commonly used for early detection of myocardial infarction.

Creatine kinase is essential for metabolism, but elevated levels may indicate the onset of ischemic disease. Our experiments show that diabetic animals have higher blood creatine kinase activity compared to conditionally healthy rats, indicating physiological changes in the myocardium during diabetes. However, in diabetic animals treated with *Sílybum mariánum*, creatine kinase levels decrease compared to the diabetic group. This reduction may indicate a lower risk of heart-related issues, muscle fatigue, and other cardiovascular diseases[26], thereby improving myocardial physiological function[27].

CONCLUSION

Our study is the first to explore the impact of *Sílybum mariánum* extract on certain physiological changes in diabetic rats. Our findings indicate that the extract enhances aortic contractility, reduces creatine kinase activity, and mitigates oxidative complications associated with diabetes. Moreover, *Sílybum mariánum* extract demonstrates significant antioxidant and antiradical properties. The observed improvements in physiological changes among diabetic rats are likely due to the antioxidant activity exhibited by the extract.

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Conflict of Interest

Authors do not have any conflict of interests to declare.

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Author's contribution

AAA carried out experiments, analyzing the results, writing an article, GUG, GSN, give advice, direction and indications for experiments, DSHA examined rat liver in vitro, DRI, SZO worked on aortic preparation, ATF head coordinator. All authors read and approved the final manuscript.

Ethical issues

None.

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