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RESEARCH ARTICLE

IN VITRO CYTOTOXIC ASSESSMENT OF CHITOSAN OLIGOSACCHARIDE LACTATE ON HUMAN BLOOD AND LYMPHOCYTE CELLS

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ABSTRACT

Chitosan oligosaccharides (ChOSs) are the reduced products of chitosan prepared by chemical or enzymatic hydrolysis. The greater solubility and low viscosity of ChOSs are of interest. The present study was the first to evaluate the toxicity of chitosan oligosaccharide lactate (ChOSlac) in human blood. For this purpose, possible oxidative effects of ChOSlac in human whole blood (hWB) and cell viability and membrane integrity effects on lymphocytes (LYMs) were evaluated in the dose range of 10-400 µg/ml and for 24 and 48 hours treatments. Firstly, total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were used to measure oxidative damage on hWB serum. Secondly, the cytological effects were evaluated using 2.3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H tetrazolium-5-carboxanilide inner salt (XTT) and lactate dehydrogenase (LDH) assays on LYMs. Exposure of cells to 10-200 µg/ml range doses of ChOSlac caused an increase in antioxidant activity and a decrease in oxidative stress but did not affect cytotoxicity. Conversely, the dose of 400 µg/ml caused a relative increase in oxidative stress and LDH leakage and decreased cell viability. In summary, ChOSlac has been evaluated positively at the specific dose range and exposure times in terms of human health as a contribution to its use in many areas such as being a biocompatible, biodegradable, and drug carrier molecule.

Keywords: Antioxidant, Chitosan oligosaccharide lactate, Cytotoxic, Human lymphocyte, Lactate dehydrogenase

1. INTRODUCTION

Chitin and chitosan are known to have significant different biological activities but low solubility makes them inconvenient to utilization in biomedicinal and food practices. They are abundant polysaccharides. Chitosan has captivated a great deal of attention due to its wide range of functions [1]. Chitosan is a linear polysaccharide comprised of deacetylated glucosamine and N-acetyl-D-glucosamine that partakes characteristics with diversified hyaluronic acids and glucosaminoglycan; whence, it is presumed that chitosan also partakes bioactivities consolidated with these compounds [2]. It can be cleaved chemically or enzymatically to procreate smaller ChOSs, which demonstrates incremented solubility. ChOSs, which has an unstable length and different degrees of deacetylation, are more robust to degradation [3]. Shorter derivatives of chitosan, ChOSs commonly have inert or anti-inflammatory characteristics and in specific biological interactions can have more affirmative features than chitosan [4]. ChOSs have demonstrated a high potency for utilization in a diversity of biomedical therapies, including anti-oxidation, antimicrobial, anti-hypertension, tissue regeneration promotion, immunostimulation, anti-obesity antitumor, calcium absorption enhancement, drug and DNA delivery enhancement, and anti-Alzheimer's disease [5]. The very good solubility and low viscosity of ChOSs at neutral pH have taken the attention of many scientists. Particularly, studies on ChOSs in nourishment fields have underlined their capacity to advance nutrient quality and human health improvement. Based on the latest interest in the biomedical trials of chitin, chitosan, and its derivatives, there are many studies that focuse on the preparation and biological effects of chitin, chitosan, CHOSs, and their derivatives. However, previous studies have not identified the biochemical and cytological effects of ChOSlac on human LYMs, such as cell viability, mitochondrial effects, antioxidant potentials, and scavenging abilities on radicals.

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If inherent defenses of the organism are suppressed by excessive production of reactive oxygen species (ROS), a condition of oxidative stress occurs, in which cellular and extracellular macromolecules can endure oxidative damage, causing cell and tissue detriment [6]. The presence of varied antioxidants in serum, plasma, and other alternative biological models makes it hard to determine each antioxidant respectively [7]. So, a few techniques have been improved to assign the antioxidative ability of diverse biological instances. The surveying of TAS can reverberate the antioxidative potential of the serum. Considering that there is a special relationship between oxidative metabolism and the effects of the applied substance, it is important to evaluate the total antioxidant capacity [8]. An automated colorimetric measurement method developed and used in many different studies provides a complete assessment for measuring TOS [9]. Preferring foods that have antioxidant properties naturally in food consumption is the most influential way of combating such as tissue damages, unwanted transformations, and prohibiting health threats [10]. Free radical production happens incessantly in all cells as part of standard cellular activity. Nevertheless, surplus free radical formation originating from endogenous or exogenous sources might play an act in numerous discomforts Antioxidants counterwork free radical-induced cell and tissue detriment by arresting the formation of radicals, scavenging them, or by assisting their decomposition [11]. In vitro cytotoxicity practices are progressively used as alternatives to all animal testing of ecological contaminants due to their reduced use of test animals, low costs, and quick performance. Over and above, in vitro models let the use of specific endpoints to state the aims of toxic impacts with ideal sensitivity and reproducibility [12]. Measuring the poisonous impacts of unknown substances in vitro by counting living cells following staining with a vital dye is an effective method. There are various methods based on cell counting with automatic counters, dyes, and cellular activity [13]. XTT assay ensures a simple use of an instrument for use with cell cultures to labor changes in the number of cells and their metabolic action**.** This assay is situated on the extracellular decrease of XTT by NADH produced in the mitochondria via an electron mediator and trans-plasma membrane electron transport. This ensures insights into cytotoxicity, cell proliferation, and cell viability[14]. The LDH assay is a means of gauging either the number of cells via aggregate cytoplasmic LDH or membrane integrity as a function of the deal of cytoplasmic LDH released into the medium [15]. This article evaluates the effect of ChOSlac on the formation of free radicals in the body, the consequences of cellular and hence tissue damage caused by free radicals as both membrane damage and cell viability, and its positive or negative effects on antioxidant defense, particularly regarding to the effect of ChOSlac on human LYMs.

ChOSs are primarily appropriate for polymer-medicament conjugates in as much as its accessibility for connection with the primary hydroxyl and amino groups of each polymer subunit and the cationic internal that authorizes ionic crosslinking [16]. The major issue of chitosan is its weak solubility at neutral pH. ChOSs, a water-soluble hydrophilic backbone was improved to accomplish these difficulties and serve as a perfect nominee for drug delivery [17]. ChOSs have received remarkable relevance as a convenient biopolymer for biomedical and pharmaceutical administrations. They can be formulated as nano-carriers for gene and drug delivery, hydrogels for tissue engineering, and membranes for hemodialysis. There are still many prospective implementations that need to be improved for ChOSlac and chitosan derivatives, showing that they have yet to be used to attain their full potency. But, the uncertain and not fully defined approach is ChOSs effects on blood cells and the immune system. This experimental research was conducted to determine the cytotoxic and oxidative effects of ChOSlac on LYMs and serum of healthy volunteer blood donors. Based on this aim, the effects of ChOSlac on cytotoxicity were firstly evaluated by applying XTT and LDH assays on LYMs. Supportively, as a comparative experiment, the role of ChOSlac on antioxidant capacity was evaluated through TAS and TOS analysis.

2. MATERIALS AND METHODS

2.1. Chemicals

ChOSlac (average Mn 5.000) and Histopaque1077 were obtained from Aldrich (Sigma, Germany). LDH cytotoxicity assay kit was provided from Cell Biolabs (CytoSelect™, US). In vitro toxicology assay XTT kit was provided from Merck (Germany). TAS and TOS kits were obtained Rel Assay Diagnostics

(Mega Tıp, Turkey). RPMI 1640 media, fetal bovine serum (FBS), antibiotic, and antimycotic solution were obtained from Gibco (ThermoFisher, Massachusetts, USA), respectively. All solutions in this study were prepared with deionized water of resistivity not less than 18.2 MΩ•cm (25°C) taken from a Milli-Q water purification instrument (Milli-Q, USA).

2.2. Whole Blood Culture and Treatment

Whole blood samples were obtained from healthy volunteer donors (n = 30). These subjects had not been on any medication for at least 4 weeks before blood sampling and they consisted of nonsmokers. Volunteers were informed according to the rules of the local ethical committee. While untreated cells are considered as a negative control (NC), other hWB cultures were treated with 10 μm ascorbic acid ($C_6H_8O_6$) and 25 μm hydrogen peroxide (H_2O_2) as a positive control for TAS and TOS, respectively. As the main study hWB cultures were treated at 10, 30, 50, 80, 100, and 400 μg/ml concentrations of ChOSlac before incubation for 24 h. Bloods provided were gathered into heparinized tubes and serum was separated from blood cells by centrifugation at 1500 × g for 10 min. Serums were run instantly or stored at -80 °C freezer. The trials were run three times independently for all analyses, and values were offered as means ± standard deviation (SD).

2.3. Lymphocyte Culture and Treatment

Blood samples were taken on the morning of the experiment, avoiding all the mentioned effects of nutritional factors and providing the necessary ethical conditions. The separated blood samples were taken in the process for XTT and LDH experiments. Separation of human blood lymphocyte cultures from hWB with Histopaque (v/v) reagent and establishment of in vitro cultures was done according to the method presented by Geyikoglu et al. [8]. The time between blood collections and establishing the cultures was less than 3 h.

Treatment groups were determined based on preliminary trials, information obtained from the literature, and kit protocols. In the first group, cells were exposed to 10 μm Mitomycin-C as a positive control for XTT and LDH tests. The second group, the negative control group not exposed to different doses of chitosan, was designed to base the rate of NAD^+ reduction background for LDH assay and standard formazan formation for the XTT assay. In the third group, cells were treated at 7 different doses of ChOSlac concentrations in the range of 10-400 μg/ml before 24 and 48 h of incubation. The trials were run three times independently for all analyses, and values were offered as means ± standard deviation (SD).

2.4. Measurement of Oxidative Stress

Antioxidant and oxidant tests were performed on serum samples taken from blood cultures for 2 h. The concentrations of these analyses at tissue level were determined with an automated assay method [18]. The Rel Assay Diagnostics kit was used to determine the levels of the parameters. TAS values were emitted as mmol Trolox equivalent/L. TOS test was set with hydrogen peroxide and the conclusions were announced in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equivalent/L). TAS and TOS activity was measured spectrophotometrically according to the manufacturer's data and with a Multiskan GO (Thermo Fisher Scientific, Massachusetts, USA).

The ratio of oxidative damage to antioxidant status in percentages provided OSI to state the intensity of oxidative stress. OSI was measured as the ratio of TOS to TAS, to the manufacturer's instructions, and expressed as a percentage. For calculation, the TAS unit obtained was converted to mmol/L and the OSI value was calculated according to the formula OSI = [(TOS/(TAS)]/10.

2.5. Membrane Integrity Evaluation

The LDH assay was performed on LYMs using LDH cytotoxicity assay kit to determine the disruption of membrane integrity at different concentrations (10-400 μg/ml) of ChOSlac. LDH activity was

measured spectrophotometrically according to the manufacturer's instructions and with an ELISA reader (Multiskan GO, Thermo Fisher Scientific, Massachusetts, US). Pre-optimized cell numbers (3×10^3 cells/ml) were seeded in a 96-well microtiter plate and a minimum of 6 wells was used for each dose at 37 °C and %5 CO_2, for 24 and 48 h. After 24 and 48 h, RPMI 1640 media was aspirated and discarded to remove the background LDH. LYMs were treated with ChOSlac doses, positive and negative controls as stated in the previous preparation stage. Following treatment, 90 µL of cell culture media from each well was transferred to a new 96-well plate followed by the addition of 10 µL LDH Cytotoxicity Assay Reagent and incubation for 30 min. The absorbance (Abs) at 450 nm was measured using a Multiskan GO. The percentage of LDH release was calculated according to the manufacturer's protocol to represent the degree of membrane integrity (Abs_{450} experimental sample - Abs_{450} negative control) / Abs_{450} positive control - Abs_{450} negative control) × 100 = %relative cytotoxicity or %LDH release).

2.6. Cell Viability Evaluation

The cytotoxic effect of ChOSlac (10-400 µg/ml) towards human LYMs was stated by toxicology assay kit XTT, which measures the metabolic activity of viable cells. This method is situated on the capability of mitochondrial dehydrogenases of cells to cleave the tetrazolium ring of XTT, yielding orange formazan crystals, which are solvable in an aqueous solution. LYMs were seeded at a deal of 1×10^4 cells in 200 µL culture medium in 96-well culture plates, a minimum of 6 wells was used for each dose and permitted to attach and grow for 24 h. The medium was then altered with 200 µL of new media containing requested doses of this method is based on the ability of mitochondrial dehydrogenases of LYMs to cleave the tetrazolium ring of XTT, yielding orange formazan crystals, which are soluble in an aqueous solution and incubated for times of 24 and 48 h. At last of the treatment, the newly prepared XTT reagent was added to each well as stated by the manufacturer Following adding the reactive solution to all of the wells, microplates were incubated for 2 h at standard terms, and then, the absorbance was measured at 450 nm using an ELISA reader (Thermo Fisher Scientific, Massachusetts, USA) with a reference wavelength of 690 nm. The cytotoxic effect of ChOSlac was expressed as a percentage of cell viability compared to untreated cells. The toxicity of ChOSlac was calculated using the formula: Absorbance of treated cells / Absorbance of untreated cells × 100 = %cell viability.

2.7. Statistical Analysis

Values of the negative control, positive control, and the treated groups were announced as the mean (± SD values) from three discrete tests. One-Way ANOVA and Fischer's least significant difference (LSD) tests were utilized to assign whether any cure significantly altered from controls or each other. Statistical determinations were made with a significance grade of 0.05. Statistical Package for Social Sciences (SPSS software, version 22.0, IBM Corporation, USA) was performed for calculations.

3. RESULTS

3.1. Oxidative Stress Activities

Significantly higher serum TAS was noticed in the four treated groups, $p < 0.05$: 30 µg/ml (+ 12.2%), 50 µg/ml (+ 14.4%), 80 µg/ml (+ 18.7%) and 100 µg/ml (+ 22.3%), is compared with the negative control. In addition, 400 µg/ml ChOSlac application decreased TAS levels by 4.3% compared to the negative control, while 10 and 200 µg/ml doses did not affect significantly.

Serum TOS of ChOSlac 30 µg/ml (- 3.1%), 50 µg/ml (- 3.7%), 80 µg/ml (- 5.3%) and 100 µg/ml (- 5%), these doses administered had significantly lower values compared to the negative control. Besides, after 400 µg/ml ChOSlac treatment, the serum TOS level increased statistically at the level of 4.1%, while the 10 and 200 µg/ml doses did not cause oxidative stress compared to the negative control.

OSI in serum markedly decreased in four treatment groups 30, 50, 80, and 100 µg/ml: 13.6, 15.8, 20.3, and 22.3% as compared to the negative control, respectively. 10 and 200 µg/ml treatment groups did not

cause any alteration in serum OSI levels. 400 µg/ml dose of ChOSlac, the serum OSI level increased in a statistically significant at the level of 8.8% (Table 1).

Table 1. TAS, TOS, and OSI results in blood cells treated with ChOSlac

Treatment		Serum TAS (mmol Trolox Eq/L)	Mean ±SD	Serum TOS (µmol H_2O_2 Eq/L)	Mean ±SD	OSI (TOS/TAS) /10	Mean ±SD
Neg c		1.39	0.18^{b}	7.55	0.96^{c}	543	69.1^{c}
Pos c		3.77^{**}	0.48^{e}	24.63^{**}	3.13^{e}	653^{**}	83.1^{e}
10	µg/ml ChOSL	1.44	0.18^{bc}	7.51	0.96^{c}	522	66.3^{bc}
30		1.56	$0,2^{c}$	7.32	0.93^{b}	469	59.7^{b}
50		1.59	0.2^{c}	7.27	0.92^{b}	457	58.1^{b}
80		1.65	0.21^{cd}	7.15	0.91^{a}	433	55.1^{ab}
100		1.7	0.22^{d}	7.17	0.91^{a}	422	53.6^{a}
200		1.42	0.18^{b}	7.62	0.97^{cd}	537	68.2^{c}
400		1.33^{*}	0.16^{a}	7.86^{*}	1^{d}	591^{*}	75.1^{d}

TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index. Neg c, negative control (untreated samples), Pos c, positive control (Ascorbic acid (10 µM) in TAS and hydrogen peroxide (25 µM) in TOS), SD, standard deviation. Values are mean ± SD (n=6); means in the figure followed by different letters (a, b, c, d) present significant differences. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control group.

3.2. XTT and LDH Assays As Indicators of Cytotoxicity

XTT assay was used to quantify cell proliferation in response to ChOSlac on LYMs at 24 and 48 h exposure times and in a 10-400 µg/ml dose range (Figure 1). No mitochondrial activity and cell viability-induced cytotoxicity was observed after 24 and 48 h of treatment with ChOSlac up to 200 µg/ml compared to the negative control. In cells treated with ChOSlac, a decrease in cell viability (9.6%, 24h, and 10.1%, 48h) was only observed at the highest tested dose (400 µg/ml).

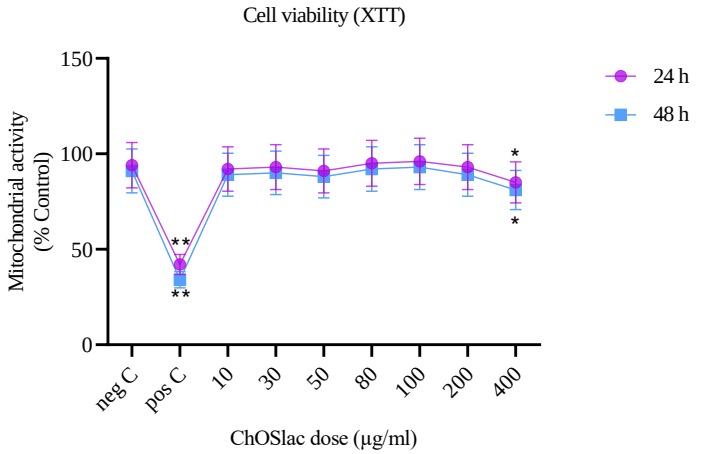

Figure 1. Effect of ChOSlac treatment on the viability of human LYMs. The cells were treated with various concentrations (10-400 µg/mL) of ChOSlac for 24 and 48 h (n=6). Percentages of viable cells after treatment were determined by XTT assay. Mitomycin C and untreated cultures were included as the negative control (neg c) and positive control (pos c), respectively. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control group.

LDH release values for membrane integrity from various doses (10-400 µg/ml) of ChOSlac at two different time points (24 and 48 h) were compared with corresponding control values (Figure 2). After incubation periods, ChOSlac produced a significant increase in LDH levels (14.9%, 24 h, and 18.6%,

48 h) versus negative controls at 400 µg/ml dose. No significant increase was obtained in LDH values for the other doses (10-200 µg/ml).

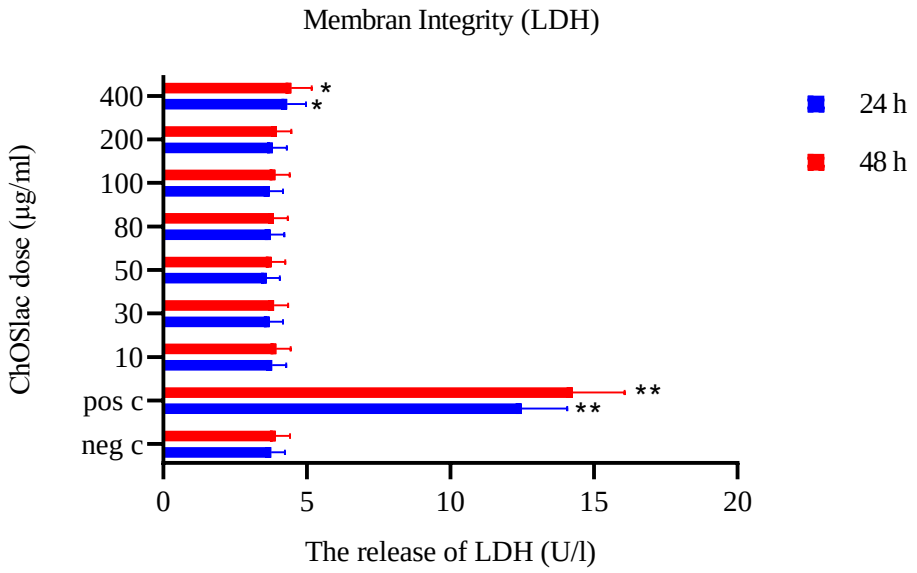

Figure 2. ChOSlac dose (10-400 µg/ml) and time-course (24 and 48 h) relationships of the membrane integrity in the in vitro lymphocyte cell culture. Lactate dehydrogenase release values of cells after treatment were determined by LDH assay. Mitomycin C and untreated cultures were included as the negative control (neg c) and positive control (pos c), respectively. The bars shown by different letters are significantly different from each other at a level of 5% (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control group.

4. DISCUSSION

Chitosan oligosaccharide (ChOS), the hydrolyzed product of chitosan, is a medley of oligomers of β-1.4-linked d-glucosamine residues and is plentiful in the exoskeleton of crustaceans and cell walls of fungi and insects [19]. ChOSs have abundantly biologic properties such as antimicrobial [20], antitumor [21], immunity regulation, and anti-apoptotic actions [22]. Currently, the antioxidant feature of COS has been a great interest because of its non-toxicity, absorption characteristics, biodegradability, and biocompatibility [23]. Hydrophobically advanced COS with cholesterol, tocopherol, alkyl groups, and deoxycholic acid can form self-assembled nanoparticles, and they can be utilized as transporters for tumor and gen-targeted drug delivery [24]. COSs have recently received remarkable interest in biomedical practices whereat this biopolymer's biocompatible, biodegradable, low-cost, abundant, and water-soluble. Besides, COSs have surprising biological activities, including antitumor, antiviral, and antimicrobial that are exciting in many areas [25]. ChOSlac has great deal of applications, such as textile finishing for water-permeable fabrics [26], drug delivery, biomedical apparatus, cosmetics, biodegradable packaging, and water treatment for organic and heavy metal contaminants [27]. ChOSlac could improve properties and could be adapted for many uses in high-throughput laboratories. Due to the widespread use of this chitosan derivative in many dose ranges and exposure times, its effect on blood cells and the immune system, which can affect human health in many ways, actually requires urgent and important research. Therefore, this study is valuable in terms of the literature and will eliminate an important deficiency.

In this study primarily, the biochemical effect of ChOSlac on blood serum was evaluated. ChOSlac application between the doses of 10-200 µg/ml (24 h exposure time) increased the total antioxidant capacity (30 µg/ml, + 12.2%; 50 µg/ml, + 14.4%; 80 µg/ml, + 18.7%, 100 µg/ml; + 22.3%) and again decreased the total oxidative stress values. Conversely, a high dose of ChOSlac (400 µg/ml) was

observed to cause a relative increase in oxidative stress (4.1%) and a decrease in the antioxidant capacity (4.3%). Moreover, the 200 μg/ml treatment group did not cause any alterations in plasma TAS, TOS, and OSI levels. This result indicated that even when used alone, ChOSlac can be used effectively to relieve stress in people taking antioxidant supplements. In other words, the formation of reactive oxygen species (ROS) stress or caused an oxidative damage of cellular and extracellular molecules and thus unwanted tissue damages. Seven et al. [28] demonstrated that the generation of exceptionally ROS plays a major role in the modulation of inflammatory effects and substantial ROS are generated throughout oxidative stages insides tissues and cells. As a defensive order counter oxidative stress, cells have multiple antioxidant molecules such as glutathione and superoxide dismutase to scavenge superoxide radicals and hydrogen peroxide. When ROS are not scavenged, these species may cause structural and functional injuries in protein, lipid, and DNA and signal transduction. The ChOSlac at these doses (10-200 μg/ml) and for 24 h treatment have ability to prevent damages on blood cells. Similar to our findings, Ngo et al. [29] revealed that administration of 10, 25, 50, and 100 μg/ml gallic acid conjugated chitooligosaccharide is a potent free radical scavenger and can inhibit oxidative damage to DNA, proteins, and lipids in chondrosarcoma cells. They also deduced that gallic acid conjugated chitooligosaccharide could increase the level of intracellular antioxidant enzymes in the oxidative stress-induced tumor induced by the Abelson murine leukemia virus (RAW264.7) cells. Similary, Lu et al. [30] stated that the incubation of mouse insulinoma (MIN6) cells with H_2O_2 dramatically increased intracellular ROS and this oxidative stress was statistically significantly suppressed by pre-treatment of sulfated chitooligosaccharides at doses of 100-500 μg/ml. Liu et al. [31] found that after the cells were incubated with ChOS (25-200 μg/ml) for 24 hours, positive effect on viability by measuring the intensity of 2.7-dichlorodihydrofluorescein (DCFH) fluorescence that a significant decrease in intracellular ROS contributed. They stated that ChOS also had inhibitory effects such as malondialdehyde in restraining the generation of lipid peroxidation and the restoration activities of endogenous antioxidants including glutathione peroxidase and superoxide dismutase with commercial reagent kits. In another aspect, one of the previous in vivo studies indicated that COS attenuates the retinal degeneration caused by oxidative stress in rats [22]. Many studies represent that ChOSlac has a substantial role in preventing oxidative stress.

The evaluation of the cytotoxic potential of ChOSlac and its comparative evaluation with oxidative stress conditions on healthy blood LYMs are of great importance in the interpretation of this biopolymer in terms of human health. This research secondly has represented that as a result of cytotoxicity analyzes performed with XTT and LDH assays, ChOSlac treatment in the 10-200 μg/ml dose range did not demonstrate any significant cytological damage in LYMs compared to the negative control. In lymphocyte cells treated with ChOSlac, a decrease in cell viability (9.6%, 24 h and 10.1%, 48 h) and an increase in LDH levels (14.9%, 24 h and 18.6%, 48 h) were only observed at the highest tested dose (400 μg/ml). There are some studies supporting this research. In one of these studies, Lu et al. [30] reported that sulfated chitooligosaccharides at different doses from 100 μg/ml to 500 μg/ml reduced cell proliferation of mouse insulinoma cells exposed to H_2O_2 by 50% as against to the control group. In another study, the protective effects of ChOS on human umbilical vein endothelial cells against oxidative damage induced by hydrogen peroxide (H_2O_2) were investigated. They found that after the endothelial cells were treated with chitosan (25-200 μg/ml) for 24 hours, there was a loss of viability in endothelial cells due to H_2O_2 (300 μM) for 12 h. They indicated that this loss of vitality was restored significantly depending on the concentration of ChOS with the MTT test [31]. In an in vivo research, Maeda and his collaborators [32] stated that 10 mg/L water-soluble chitosans increase the activity of natural killer cells, which are important elements of innate immunity, in intraepithelial and splenic LYMs.

An in vitro experiment was conducted in this research, that's why it is significant to evaluate the possible result of observed alterations for the human body. But, this is a prevalent issue in experimental toxicology. The results of this research only show a direction for further studies, and these results are not sufficient to reach a definitive result about the practical use of ChOSlac in medicine, pharmacy, or

a specific area of use. In addition to the results supporting this research in vivo or different cell culture studies, different evaluations can be made. To criticize our study, while the concentration of chitosan determined as cytotoxic for lymphocyte cultures in our study was 400 μg/ml, it was stated as above 2000 mg/kg/day for chitooligosaccharide in a different in vivo study. In the research mentioned, Kim et al. [33] conducted a subacute oral toxicity study of ChOS in Sprague-Dawley rats. They administered 500, 1000, and 2000 mg/kg/day ChOS by gavage to rats for 4 weeks, respectively. Hematological, biochemical, and histopathological examinations were performed in addition to body weights. As a result, they stated that the subacute toxicity of ChOS was low and the level of unobserved side effects was above 2,000 mg/kg in rats. When comparing cell culture and in vivo, and considering that we use different types of chitosan, this study seems to support my work. In another in vivo study, Mei et al. [34] indicated that treatment with chitooligosaccharide at a dose of 500 or 1000 mg/kg body weight resulted in higher phagocytic activity than the control level. They stated that at these concentrations chitooligosaccharide increased macrophage phagocytic activity. In another in vivo study that parallels this research, Yeh et al. [35] investigated the impact of chitosan on the immune responses and LDH levels in mice myelomonocytic cell line WEHI-3. Mice were disunited into control, chitosan (5 and 20 mg/kg) treated, and acetic acid (vehicle) treated groups. Mice's blood samples were evaluated for cell markers, natural killer cell proliferation was studied by flow cytometry. Administration of chitosan at the indicated doses (5 and 20 mg/kg) did not alter the cytotoxic activity of T, B, and natural killer cell proliferation. It has been noted that chitosan increased the white blood cell quantities but did not alter LDH activity in leukemia mice. As the last comment, they stated that chitosan may support in futurity works on the refinement of immune responses in the remedy of leucaemia. In a study supporting this study evaluating the oxidative stress-induced cytotoxic effect, Khodagholi et al. [36] demonstrated that chitosan arrests oxidative stress-induced amyloid-β creation and cytotoxicity in NT2 neurons via mediating Nrf2 and NF-κB pathways. In this study, the toxicity of ChOSlac, which is used in many areas such as biomedical devices, water treatment for organic and heavy metal contaminants, textile finishing for water-permeable fabrics, biodegradable packaging, cosmetics, was evaluated in terms of human health.

5. CONCLUSION

This experimental study comparatively evaluated the biochemical and cytotoxic effects of ChOSlac on the blood serum and LYMs of healthy volunteer blood donors. ChOSlac with the doses in the range of 10-200 μg/ml did not cause any cytotoxic damage due to mitochondrial activity and membrane damage and did not affect cell viability. Moreover, it led to a remarkable increase in the antioxidant capacity of serum. On the other hand, ChOSlac with the high dose of 400 μg/ml was found to relatively increase in the oxidative stress for 24 and 48 h treatment groups while it caused a slight damage of cellular membrane and slightly altered mitochondrial activity.

Collectively, ChOSlac can be used as a radical scavenger to prevent damage to the cellular organizations, and as a structure and symptom-modifying agent in these areas and many treatments. The findings of this research revealed that ChOSlac can be used in human health, as biocompatible, biodegradable and drug carrier molecule and can be a good alternative to other chitosan derivatives.

CONFLICTS OF INTEREST

The authors have no potential conflicts of interest for the research, authorship, and/or publication of this article.

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