ATTENUATION OF UVC-INDUCED TOXICITY BY *Linum usitatissimum* L OIL IN RATS: MONITORING OF SOME HEMATOLOGICAL AND BIOCHEMICAL CONSTITUENTS

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ABSTRACT

The aim of this study was to investigate the protective effect of *Linum usitatissimum* L. seed oil (LUSO) against ultraviolet C (UVC) exposure through monitoring some hematological and biochemical constituents of rats. For this purpose, rats were divided into three groups: control, UVC and UVC+LUSO. The rats of UVC and UVC+LUSO groups were subjected to UVC during 1 h twice a day for 4 weeks. LUSO (4 ml/kg b.w.) was given to rats of UVC+ LUSO group by gavage before each irradiation period. Results showed that lipase and cholesterol levels were markedly higher in UVC group than control. Lipase decreased significantly, and cholesterol diminished slightly with combined treatment of UVC and LUSO. While the level of red cell distribution width (RDWc) decreased in UVC group, it increased significantly after LUSO supplementation. Apart from that, calcium and monocyte levels increased markedly in UVC+LUSO group compared with control and UVC groups. Significant decreases were determined in amounts of white blood corpuscles and lymphocytes as well as an increase was observed in monocyte percentage of UVC+ LUSO group compared to control. This investigation showed that treatment of LUSO with UVC supplied relative protection against UVC injury and perfected some blood parameters significantly, even when compared with control group.

KEYWORDS: Ultraviolet C; *Linum usitatissimum* L; hematological and biochemical constituents; rat

1. INTRODUCTION

Ultraviolet radiation C (UVC) (200-280 nm) which has the most powerful and dangerous light among the UV light spectrum [1] cannot reach the earth surface due to its absorption in the ozone layer of the atmosphere under normal conditions [2]. However, Osman et al. [3] reported the presence of UVC on biosphere and linked this circumstance to ozone depletion. In addition, UVC has been used more frequently in the laboratories in recent years, due to its sterilizing and biocidal properties [4]. Because of these reasons, detrimental effects of UVC on humans have appeared increasingly. For example, escalations in skin cancers related to UVC have been reported [5].

The skin is the first and main organ [6] suffering from UV produced by sun and artificial sources. Later, blood and other organs are presumably affected from damage of skin. UV-induced oxidative stress (OS) was reported in some previous studies [7-10]. It was reported that OS led to alterations in different physiological and biochemical systems of some animals [11, 12]. Parameters of these systems have essential importance in the physiopathological evaluation of animals [13].

Oral or topical application of botanical compounds that have photoprotective features is one of the approaches to protect the UV-induced damage. *Linum usitatissimum* L. seed oil (LUSO) is a generous source of alpha-linolenic acids and lignans that are regarded as the biologically active compounds supplying useful influences [14-16]. Lignans have been indicated to possess antioxidant properties [17-19]. To the best of our knowledge, this is the first study investigating preventive effect of LUSO against UVC exposure through monitoring some hematological and biochemical variables.

The aim of the current study is to clarify the negative effects of UVC on several blood variables of albino rats, and find out the supposed positive act of LUSO in attenuation of these effects.

2. MATERIALS AND METHODS

2.1. Determination of LUSO dose

LUSO, consisting of palmitic acid 6.0 %, stearic acid 2.5%, arachidic acid 0.5%, oleic acid 19.0 %, linoleic acid 24.1%, alpha-linolenic acid 47.4%, and other acids 0.5%,

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was obtained from a herbal store in Sanliurfa, Turkey. In this study, its dose was determined as 4 ml/kg body weight (b.w.) according to Bhatia et al. [20].

2.2. Animals

Rats (Sprague-Dawley male albino) weighing 235-285 g were provided from the animal house of Firat University and housed in plastic cages (7 rats per cage). They were fed with wheat-soybean-meal-based diet and water *ad libitum* in stainless cages, and received human care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institutes of Health [21]. Rats were shaved with a safety-razor, and this process was repeated before each UV exposure period. They were kept at 20±2 °C daily in light/dark cycle.

2.3. Ultraviolet radiation

In the current study, UVC light was produced by a lamp (Mazda TG model) with 30 Watt power and 90 cm length. Rats were exposed to UVC in a 182x68x50 cm glass box as described by Brainard et al. [22] and Turker [10]. The UVC lamp was attached on the lid of the box in a distance of 44 cm to the rats. The animals were subjected to UVC light radiated from the lamp for 28 days. The intensity of the UVC was determined by a spectrophotometer (wavelength with a peak value: 254 nm). In this experiment, energy of the UVC light emitted from the lamp per cm^2 for one second was found to be 0.0014 joule/cm^2. This amount was adjusted according to Turker [10].

2.4. Experimental design

The animals were divided into three groups. 1) Control group: received 1 ml normal saline (0.9 %) by gavage during 28 days; 2) UVC group: exposed to artificial UVC irradiation (1.25 mW/cm^2) during 1 h twice a day and receiving 1 ml normal saline (0.9 %) by gavage for 4 weeks; 3) UVC+LUSO group: receiving 1 ml LUSO (4 ml/kg b.w.) by gavage and exposed to UVC light during 1 h twice a day for this time.

2.5. Analysis of biochemical and hematological parameters

At the end of the treatments, rats were anesthetized with ketamine (100 mg/kg ip) [23]. Blood samples were withdrawn from the cardiac puncture by using an injector for the measurement of hematological and biochemical constituents under light anesthesia; then, rats were euthanized with inhalation of CO_2. Blood specimens were drawn instantly into clot activator tubes. The serum samples were obtained to measure biochemical constituents by centrifuging blood samples at 3,000 rpm for 15 min at 4 °C. For hematological parameters, the blood samples were drawn into tubes with EDTA. Biochemical parameters including aspartate aminotransferase (AST) (EC 2.6.1.1), alanin aminotransferase (ALT) (E.C 2.6.1.2), lipase (EC 3.1.1.3), creatine phosphokinase (CPK) (EC 2.7.3.2), alkaline phosphatase (ALP) (EC 3.1.3.1), gamma glutamyl transferase (GGT) (EC 2.3.2.2), urea, creatinine, total protein, albumin, triglyceride, cholesterol, high density lipoprotein (HDL), total bilirubin, direct bilirubin, indirect bilirubin, glucose, potassium (K), chloride (Cl), calcium (Ca), rheumatoid factor-II (RF-II), C-reactive protein (CRP), unsaturated iron-binding capacity (UIBC) and antistreptolysin O (ASO) were analyzed by an autoanalyzer (Roche, Cobas Integra 400 plus) using commercial kits. Hematological parameters including red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), red cell distribution width (RDWc), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), percentage of lymphocytes (LY%), percentage of monocytes (MON%), percentage of granulocytes (GR%), mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDWc), lymphocytes (LY), monocytes, granulocytes (GRA), white blood corpuscles (WBC) and mean corpuscular hemoglobin (MCH) were measured by a hematological assay apparatus (Coulter, Beckman).

2.6. Analysis of Data

All data were presented as means ± standard deviation (SD). The statistical analyses were realized using the Minitab 13 for windows package program. Means and standard deviations were measured according to the standard methods for all parameters. One-way analysis of variance (ANOVA) statistical test was used to determine the differences between means of the experimental groups accepting the significance level at $p< 0.05$.

3. RESULTS

3.1. Effect on hematological constituents

While level of RDWc decreased in UVC group compared to control, its level increased significantly after LUSO supplementation. Monocyte level increased markedly in UVC+LUSO group comparing to both control and UVC groups. Furthermore, significant reductions of WBC and LY levels and escalation of MON% were determined in UVC+LUSO group compared with control (Table 1).

3.2. Effect on serum enzymes

The activity of lipase increased in UVC group compared to control, but it was decreased markedly with LUSO supplementation (Table 2).

3.3. Effect on lipid profile

Cholesterol level was significantly higher in UVC group than control (Table 3).

3.4. Effect on other biochemical parameters

Calcium level of UVC+LUSO group increased significantly when compared to both control and UVC groups (Table 4).

TABLE 1 - Effect of UVC on hematological parameters of rat (means ± SD).

Parameters	Control	UVC	UVC+LUSO
RBC 10^6/mm^3	4.76±0.46	5.03±0.78	5.25±0.85
HGB gr/dl	14.08±0.50	14.40±0.45	14.80±0.76
HCT %	48.88±1.68	50.02±1.85	51.56±3.39
MCV μm^3	60.90±2.59	61.96±2.10	59.58±3.21
RDWc %	14.98±1.33	13.28±0.63[a]	16.10±1.33[b]
MCHC gr/dl	28.80±0.34	28.78±0.61	28.70±0.58
PLT 10^3/mm^3	627.40±150.35	731.20±87.19	663.60±120.68
LY %	90.54±5.91	92.00±2.97	84.62±10.09
MON %	4.82±0.89	4.56±1.57	6.56±1.27[a]
GR %	3.22±0.94	3.72±1.08	3.22±0.23
MPV fL	6.80±0.62	6.40±0.29	7.08±0.61
PCT %	0.42±0.07	0.47±0.06	0.47±0.07
PDWc %	17.12±1.15	17.58±1.10	16.82±0.83
LY 10^3/mm^3	12.92±1.05	11.22±2.28	10.06±1.38[a]
MONO 10^3/μl	0.55±0.14	0.54±0.17	0.84±0.21[a,b]
GRA 10^3/μl	0.42±0.16	0.42±0.13	0.47±0.08
WBC 10^3/mm^3	13.86±1.20	12.18±2.22	11.30±1.67[a]
MCH pg	17.54±0.85	17.82±0.59	17.08±0.70

Values are given as means ± SD; [a] Control versus treated $p< 0.05$ level; [b] UVC versus treated p< 0.05 level.

TABLE 2 - Effect of UVC on serum enzymes of rats (means ± SD).

Parameters	Control	UVC	UVC+LUSO
AST U/L	191.40±61.62	206.40±27.79	211.80±94.27
ALT U/L	70.80±16.69	87.80±12.52	99.60±37.22
LIPASE U/L	4.76±0.36	4.94±0.34[a]	4.14±0.32[b]
CPK U/L	699.80±42.85	613.40±72.31	630.80±107.20
ALP U/L	220.20±36.29	205.40±69.58	203.20±70.53
GGT U/L	1.06±0.21	1.14±0.61	1.11±0.36

Values are given as means ± SD; [a] Control versus treated $p< 0.05$ level; [b] UVC versus treated $p< 0.05$ level.

TABLE 3 - Effect of UVC on serum lipid profile of rats (means ± SD).

Parameters	Control	UVC	UVC+LUSO
Triglyceride mg/dl	66.20±11.19	61.00±16.96	75.00±14.09
Cholesterol mg/dl	62.40±5.32	69.20±2.17[a]	65.80±3.90
HDL mg/dl	17.80±3.27	17.40±4.72	15.00±2.00

Values are given as means ± SD; [a] Control versus treated $p< 0.05$ level; [b] UVC versus treated $p< 0.05$ level.

TABLE 4 - Effect of UVC on some biochemical parameters of rat (means ± SD).

Parameters	Control	UVC	UVC+LUSO
Urea mg/dl	47.80±4.44	44.00±5.15	41.20±5.40
Creatinine mg/dl	0.31±0.06	0.30±0.03	0.34±0.04
T. protein mg/dl	7.76±0.73	7.54±0.22	7.62±0.40
Albumin g/dl	3.62±0.18	3.78±0.33	3.60±0.52
T. bilirubin mg/dl	0.07±0.02	0.08±0.02	0.07±0.02
D. bilirubin mg/dl	0.03±0.01	0.03±0.01	0.03±0.01
I. bilirubin mg/dl	0.04±0.01	0.05±0.01	0.04±0.01
Glucose mg/dl	194.20±33.98	191.80±26.66	196.80±5.54
K (mmol/L)	5.32±0.36	5.62±0.74	5.40±0.72
CL (mmol/L)	96.20±2.39	96.40±1.67	97.60±2.07
Ca (mg/dL)	9.78±0.61	10.18±0.29	10.70±0.19[a,b]
RF-II (IU/mL)	9.30±1.54	8.52±2.26	9.16±2.97
CRP (mg/L)	0.20±0.06	0.21±0.07	0.19±0.07
UIBC (ug/dL)	449.80±79.57	437.80±44.82	453.00±25.54
ASO (IU/mL)	9.00±1.22	10.40±2.97	11.20±1.92

Values are given as means ± SD; [a] Control versus treated $p< 0.05$ level; [b] UVC versus treated $p< 0.05$ level.

4. DISCUSSION

Ozone layer is a rather minor and important component of the atmosphere. It makes life sustainable on biosphere through eliminating the harmful UV radiations. Currently, escalated phototoxicity in atmospheric UV radiation has been claimed after destruction of ozone [24, 25]. The skin is the most suffering organ from UV. Hazardous compounds occurring after UV-induced skin cell injury may leak into the circulation system. Therefore, we researched protective effect of LUSO against UVC radiation by monitoring some blood parameters.

No significant alteration was observed in terms of hematological parameters between UVC and control groups. Significant decreases of WBC and LY constituents were observed in UVC+LUSO group compared to control (Table 1). These decreases may be attributed to appeased immune response, or LUSO supplementation may have caused infiltration of these parameters into tissues. According to Record and Dreosti [26], UV irradiations resulted in increased neutrophil immigration to dermal layer of skin.

As shown in Table 1, while level of RDWc decreased in UVC group markedly, it increased dramatically in UVC+ LUSO group. Monocyte level increased significantly in UVC+LUSO group compared with both control and UVC groups. Accordingly, MON % value of UVC+LUSO group was markedly higher than that of control group. LUSO may have protected RBC and monocyte membranes against UVC phototoxicity; therefore, degradations of old RBC and monocytes might have been prevented in UVC+LUSO group. These results may be associated with increased heterogeneous dispersion of erythrocyte dimensions and monocytes. Thus, RDWc and monocyte levels may have increased in this group. Our findings are in accordance with Rifkind et al. [27].

In the present trial, the activity of lipase was increased in UVC group compared to control, but it was decreased markedly with LUSO supplementation (Table 2). Möller et al. [28] reported lipase inhibitory effects of different plant extracts including seed extracts of *Linum usitatissimum*. They suggested that high fat ingredients of seeds gave rise to lipase inhibition. Accordingly, we can say that inhibition of lipase may have occurred in UVC+LUSO group owing to LUSO. According to our results, no significant alterations of AST, ALT, CPK, ALP and GGT levels were recorded between groups. However, Altinas et al. [12] reported increased ALT, AST and decreased GGT, ALP levels after administration of UVC in mice. This discrepancy may be related to different doses and length of time of UVC application as well as animal models.

Serum cholesterol level is one of the indicators of liver function. It can be accepted as an index of the balance between hepatic cholesterol synthesis and cholesterol catabolism process. In this study, cholesterol was increased markedly in UVC group compared to control (Table 3). UVC may have caused proportional inadequacy in cholesterol catabolism. Similar to our study, artificial UVC administration increased cholesterol levels of mice (Altinas et al. [12]). We determined slightly decreased cholesterol level in combined treatment of UVC and LUSO, suggesting that LUSO might have prevented the development of hypercholesterolemia because of its lignan and alpha-linolenic acid content [18, 19, 29]. Apart from that, high calcium levels of UVC+LUSO group might have caused reduction of cholesterol [30].

Calcium level in UVC+LUSO group was increased significantly compared to control and UVC groups (Table 4). Treatment with LUSO may have given rise to this increase owing to elevated intestinal absorption or reduced renal loss as observed by Zhang et al. [31] who investigated the effect of *Ligustrim lucidim* A. on calcium.

The results showed that analyses of blood parameters play an important role to detect physiological changes and general health status of rat exposed to UVC. Our trial demonstrates that UVC irradiation influences several blood constituents in circulation system, and alters proportionally their levels. Besides, we thought that LUSO has the relative protective potency against impact of UVC irradiation.

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