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Evaluation of the chemical composition, genotoxic and cytotoxic effects of cocklebur (*Xanthium strumarium* L.) seed oil on human blood cells

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Abstract
The cocklebur (*Xanthium strumarium* L.), is a wild plant, plant's seed contains up to 25% crude oil. It is obvious that the oil rate can be increased if this plant is breeding and cultivated. This study will evaluate whether this plant can be cultivated and used as an edible oil plant. In this study, cocklebur seeds were collected from nature and grown under cultural conditions. The oil was obtained from cold press, and different doses of oils were determined to malondialdehyde (MDA) and superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and acetylcholinesterase (AChE) activities in human erythrocytes. Its effects on mitotic index (MI), micronucleus (MN) and replication index (RI) parameters in human peripheral lymphocyte cultures were examined. According to the results, seeds of cocklebur averaged 30% oil content. The highest fatty acid composition was linoleic acid at 72.624%. The genotoxicity results showed a genotoxic effect to the dose-dependent (0.16, 0.32, and 0.50 µL mL^{-1}). Cocklebur seed oil caused significant decreases in MI and RI values (0.16, 0.32, and 0.50 µL mL^{-1}). Afterwards, MDA level increase, SOD, CAT, GST, GPx and AChE activities of human blood cells significantly decreased in increasing doses of oil compared with not-treated cells, especially from the 0.08 µL mL^{-1}. Consequently, cytotoxic and genotoxic effects are detected in high concentrations, cocklebur oil can be evaluated at lower doses. Antioxidant results also support this opinion.

Key words
Xanthium strumarium, fatty acid, genotoxicity, cytotoxicity

Introduction
Vegetable oils can be used both as a nutrient source and for industrial purposes (Ardabili et al. 2011). Also, they have an important role in human healthy as well as in human nutrition. Oils, which are a major metabolite such as carbohydrates and proteins, are an important food source for humans and animals. Seeds and fruits of oil crops, which can benefit economically, contain a high amount of oil. Cocklebur (*Xanthium strumarium* L.), which belongs to the Asteraceae family, is seen as weed in many parts of the World. Cocklebur, which can be used in many areas from traditional medical treatment to modern pharmacology, can be recommended such as an alternative oil crop. It is commonly growing in marginal areas (warm, cold, and arid) and it is an annual herb, up to 1 m in height, with a short, stout, hairy stem (Ruan et al. 2012). The plants fruit are 1.0-3.5 cm long, brown, hard, woody hook-shaped and thorny. Each fruit contains two seeds (Eymirli and Torun 2015). Its seed contains 25% to 40% crude oil, which is approximately 77% linoleic acid (Chang et al. 2013), and it has important potential in terms of fatty acids compositions. Fatty acid composition of cocklebur is similar to sunflower oil (Cesur et al. 2018). Standard safflower oil contains about 6–8 % palmitic acid, 2–3 % stearic acid, 16–20 % oleic acid and 71–75 % linoleic acid. The vegetable oil ratio and fatty acid composition obtained from the oil crops are very important, because they determined their economic importance. Not only physical and chemical properties of oils but also plants growing condition are very important for oil quality. For example; Plants are synthesized less linoleic acid but more oleic acid with increasing temperatures. In cold climates and high altitudes, mostly unsaturated fatty acids are formed (Samancı and Özkaynak 2003, Uppstrom 1995). There is a difference among species in regards to fatty acid composition, moreover intraspecific variation can be shown (Linoleic type sunflower, oleic acid type sunflower) (Karaca and Aytaç 2007). Olive, peanut, coconut and sesame are important for human nutrition and these vegetable oils are rich in oleic and linoleic acid (Baydar and Erbaş 2000). Another fatty acid can be found in vegetable oils is erucic acid ($C_{22:1}$). It has been determined that erucic acid has undesirable effects on muscles, heart and animals' growth. The World Health Organization (WHO) determined the rate of erucic acid in edible oils up to 5%. Cocklebur oil does not contain erucic acid (Tosun and Özkal 2000, Zhu et al. 2006). Cocklebur oil fatty acid composition has properties close to edible oils.

On the other hand, non-edible oils can be used in other industrial area, such as biodiesel production, oils, which are containing linolenic acid, dry quickly so they are widely used in the production of polishes, paints and varnishes.

Different studies record the genotoxic and cytotoxic potentials of oils and extracts (Fan et al. 2019, Zor and Aslan 2020). However, lack of a systematic model to determine these potentials makes it difficult to make decisions with reliable results. The cytokinesis-block micronucleus assay, recently approved by the OECD (2016), is consented one of the most reliable methods for evaluating genotoxicity and cytotoxicity with many biomarkers. The biomarkers are mitotic and replication indexes (proliferative capacity in human lymphocytes), apoptosis, necrosis, and cytostasis percentage for cytotoxicity (Fenech et al. 1999) and micronuclei (clastogenic and aneugenic effects), nucleoplasmic bridges, and nuclear buds for genotoxicity (Fenech 2007).

According to the literature, the vegetable oil and fatty acids composition obtained from cocklebur seeds can be evaluated economically. Also, accordingly fatty acid composition of cocklebur oils, are used in different areas such as industry, medicine, pharmacy and herbal medicine (Uskutoglu 2018). In this study, the fatty acid composition and mineral matter of vegetable oil from cocklebur (*X. strumarium* L.) seeds, which was grown under cultural conditions were investigated, different doses of this oil were evaluated to malondialdehyde (MDA) and superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and acetylcholinesterase (AChE) activities in human erythrocytes. Also, its effects on mitotic index (MI), micronucleus (MN) and replication index (RI) parameters in human peripheral lymphocyte cultures were examined.

Materials and Methods

Plant material

The seeds of cocklebur were collected from Muslubelen (1440 m), -Yozgat, Turkey, on 16 September 2014. The seeds of cocklebur were sown (60 cm × 20 cm) in Yozgat (Topçu), Turkey, at the end of March 2016. When the plants had 3 or 4 leaves, N (150 kg ha^{-1}) was applied to the soil. After the soil was irrigated once. When fruits were mature, cocklebur plants were harvested. The plants are stored at room temperature (25°C), in the shade away from direct sunlight. Then, seeds obtained for the cold press analysis and obtained oil stored at refrigerator (+4°C).

Soil data

The physicochemical properties in experimental area's soil surface layer (at 0–30 cm) are presented in Table 1. According to the Table 1, it can be seen that the soil of the experimental area contained a medium level of organic matter (2.49%). The soil of the trial area has a loamy texture.

Table 1. Some soil properties in experiment area

Properties	Values	Class	Properties	Values	Class
Texture class	Loamy	-	P_2O_5 (µg g^{-1})	78	High
pH	7.09	Neutral	K_2O (µg g^{-1})	728	High
Salinity (%)	0.178	Low	Ca (µg g^{-1})	7060	High
$CaCO_3$ (%)	7.15	Medium lime	Cu (ppm)	2.84	Enough
Total N (%)	0.15	Enough	Mg (µg g^{-1})	5604	Very low
Organic matter (%)	2.49	Medium	Fe (ppm)	8.08	High
Mn (µg g-1)	4.07	Low	Zn (ppm)	0.62	Low

Analysis of the fatty acid composition

Pre-treatment: Fatty acids methylation: 0.1 g of oil was taken and put into a 15 mL tube with a cap. Add 1 mL of 2 N methanolic KOH solution and vortex for 2 minutes. After waiting 15 minutes, 10 mL of hexane was added and mixed thoroughly. It was centrifuged at 7000 rpm for 10 minutes. 1 microliter of the upper phase was injected into the GC device. After the fatty acids were methylated, they were analyzed by Shimadzu Gas Chromatography (2025) with Flame Ionizer Detector (FID). Teknokroma brand TR-CN100 column of 60.0 m × 0.25 mm × 0.20 µM is used. The column heat was held initially at 80 °C for 2 min after injection, then increased to 5 °C with 140 °C/min heating ramp for 2 min and increased to 240 °C with 3 °C min^{-1} heating ramp with 5 more minutes, and the injector temperature was set to 240 °C. The total analysis time is 61 minutes. The carrier gas was helium with column flow rate of 30 mL min^{-1}. The gas flows used were determined as H2 = 40 mL min^{-1} and dry air = 400 mL min^{-1} (AOAC 1990).

Determination of nutrient contents

The heavy metals and nutrients in the samples were detected using an iCAP-Qc ICP-MS spectrometer (Thermo Scientific) at the Yozgat Bozok University, Science and Technology Application and Research Center. ICP-MS conditions were listed as follows: The calibration curve was made between 0.1 ppb and 500.0 ppb for the relevant elements, totaling of 7 points. The dilutions of the solutions to be used to draw the calibration curve were made with a 3% nitric acid solution prepared with ultra-distilled water. Plasma power 1450 W, Nebulizer Pressure to 3.01 bars; Spray Chamber Temperature was measured as 2.8 °C. High purity argon gas was used as the gas; plasma gas was set at 0.7 L min^{-1}, Nebulizer gas at 0.9 L min^{-1}. The measurement time was made in 0.01 seconds. The samples were measured 3 times in total (Yetim 2002).

Obtaining of erythrocytes

20 mL blood samples were taken from heparin tubes from 6 healthy male volunteers who did not use cigarettes and alcohol and were not exposed to any chemicals in their workplace. Heparinized whole blood was centrifuged at 2000 rpm for 10 minutes and the supernatant was removed. Then, erythrocytes were prepared by washing three times with phosphate buffer (pH 7.4). The hemoglobin concentration was determined according to the Drabkin (1946) method.

Obtaining of leucocytes

6% dextran prepared in isotonic saline was added to blood samples taken from 6 healthy male volunteers who did not use cigarettes and alcohol and were not exposed to any chemicals in their workplace, and they were subjected to a waiting period of 30 minutes. The upper layer was taken up in another tube containing 2.25 mL EDTA and centrifuged at 1000 rpm. Then leukocytes were prepared by washing with Tris buffer.

Obtaining of lymphocytes

5 mL blood samples were taken from heparin tubes from 6 healthy male volunteers who did not use cigarettes and alcohol and were not exposed to any chemicals in their workplace. The samples were collected into Vacutainer tubes with anticoagulant heparin (Becton Dickinson USA).

Groups and applications

The protocol applied in this study was countenanced by the Yozgat Bozok University Ethics Committee with a protocol number (2017-KAEK-189 2018.12.12 11). For this study, 9 groups (6 samples in each) were composed.

These groups were:
Control group (no oil application was done)
0.01 µL mL^{-1} *X. strumarium* seed oil treated group
0.02 µL mL^{-1} *X. strumarium* seed oil treated group
0.04 µL mL^{-1} *X. strumarium* seed oil treated group
0.08 µL mL^{-1} *X. strumarium* seed oil treated group
0.16 µL mL^{-1} *X. strumarium* seed oil treated group
0.32 µL mL^{-1} *X. strumarium* seed oil treated group
0.50 µL mL^{-1} *X. strumarium* seed oil treated group
1 µL µL mL^{-1} *X. strumarium* seed oil treated group

The oil was added to the cells and left for 30 minutes incubation at 37 °C. Hemolysate was obtained from cells waiting at -20 °C until working time with hypotonic sodium phosphate buffer pH (7.4). The activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), acetylcholinesterase (AChE) and malondialdehyde (MDA) levels from the hemolysate samples compared to the control group (UV-VIS Spectrophotometer (Biotech Engineering/ Spectroscan 60 DV)) were determined.

Measurement of MDA levels

MDA forms a pink colored complex as a result of incubation with TBA at 90 °C under aerobic conditions. The absorbance of this complex is determined on the spectrophotometer at a wavelength of 532 nm. The amount of MDA, the final product of LPO, which reacted with thiobarbituric acid (TBA), was measured using the method of Ohkawa et al. (1979). The absorbance of the mixture with TBA was determined by the spectrophotometer.

Antioxidant enzyme assays

Habig et al. (1974)'s method was used in determining GST activity. The enzyme activity was determined at 340 nm by the GST enzyme conjugated with 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), based on the oxidation of GSH.

In SOD determination, by using the method of Marklund and Marklund (1974), the absorbance increased by autooxidation of pyrogallol at 440 nm in alkaline medium was measured. Firstly, Tris-EDTA buffer and different volumes of supernatant were added to the cuvettes, and then enzyme source was added to them. Then, pyrogallol was added to these mixtures and absorbance was measured at 440 nm in the spectrophotometer.

CAT activity was determined by the method revealed by Aebi (1984). In the first stage, Triton X-100 was added to the supernatant to reveal CAT in peroxisomes, and then dilution was made by adding phosphate buffer. Then H_2O_2 was added for starting the enzymatic reaction and the absorbance measured at 240 nm. The method of Paglia and Valentine (1967) was applied to determine GPx activity. This method is based on the principle of measuring the absorbance created by GR oxidizing nicotinamide-adenine-dinucleotide hydrogen phosphate (NADPH) at 340 nm. The oxidation of NADPH to Nicotinamide-adenine-dinucleotide phosphate (NADP) causes a decrease in absorbance at 340 nm, thus indirectly used to determine the activity of GPx. Enzymatic reaction was started by adding H_2O_2 on this mixture and absorbance values were read at 340 nm for 3 minutes.

Measurement of AChE activities

Cholinesterase catalyzes the breakdown reaction of acetylcholine to thiocholine and acetate. As a product, 5-thio-2- nitrobenzoic acid, which gives yellow color, is formed as a result of the reaction of DTNB with the released thiocholine. The intensity of the resulting color was measured at 412 nm according to Ellman et al. (1961) method. Firstly, Na-K Phosphate Buffer (pH=8.0), sample, etopropazin and DTNB were placed in spectrophotometer cuvettes. At the end of the incubation period, absorbance measurement was made by adding acetylcholine iodide.

Peripheral lymphocytes culture

Culture tubes consisted of 5 mL peripheral blood karyotyping medium (Biological Industries, Israel) and 0.4 mL blood. Lymphocytes were incubated at 37 °C and 5% CO_2 for 72h. The oil was added to the tubes in different

concentrations added at the 24th hour. After 44h incubation times, cytochalasin-B (Sigma-Aldrich, Germany) was added to micronucleus tubes for cytokinesis block. In harvesting, cells were treated by hypotonic solution (0.075 M KCl) and several times fixed in fixative solution (methanol: acetic acid, 3:1, v v-1). In preparation, 3 to 4 drops of cell suspension were dropped onto the slide. Slides were stained with Giemsa (Merck, Germany) (Gümüş et al. 2020).

Microscopic analyses

2000, 500, and 1000 cells were counted in MI, RI and MN evaluation, respectively. The formulae are given below.

MI (%) = (Cells in the metaphase / Total cells) × 100

RI = [(1 × mono-) + (2 × di-) + (3 × tri-) + (4 × polynucleate cells)] / Total cells

MN (%) = (Number of micronucleus / Total cells) × 100 (Gümüş et al. 2020).

Statistical analyses

The data obtained in the study were evaluated by using One Way Analysis of Variance (ANOVA) and Tukey test in Windows SPSS 20.0 computer program. P <0.05 value was considered statistically significant. Data were signified as the means ± standard deviation (S.D).

RESULTS AND DISCUSSION

Fatty acid composition of the cocklebur seeds

In this study, seeds of cocklebur averaged 30% oil content. Other studies showed that cocklebur oil content can reach up to 37-42% (Klimakhin et al. 2015, Rozina et al. 2017). Fatty acid compositions of cocklebur oil are given in Table 2. Fatty acids determined in oil obtained from cocklebur seeds were palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), linolenic acid ($C_{18:3}$), arachidic acid ($C_{20:0}$), and gondoic acid ($C_{20:1}$). The highest fatty acid component was linoleic acid ($C_{18:2}$) with 72.624%, followed by oleic acid ($C_{18:1}$) with 16.87%. According to other studies, the linoleic acid content was determined as 76.97% (Cesur et al. 2018, Coşge Şenkal et al. 2019). Also, cocklebur seed-oil were consisted of saturated palmitic (5.59%) and stearic acids (2.14%); monounsaturated oleic acid (20.07%); and the more valuable polyunsaturated linoleic (68.06%). Our results showed similarities to the other studies (Klimakhin et al. 2015, Coşge Şenkal et al. 2019). The linoleic acid content (72.62%) was close to safflower and sunflower oils, but higher than soybean, olive and peaunt oils (Coşge Şenkal et al. 2019, Gursoy 2019). Chemical and physical properties of oils can change with their fatty acid composition. Saturated and unsaturated fatty acids are found in all vegetable oils and their ratios can be changed (İmer and Taşan 2018, Gursoy 2019). Clinical observations established a 1:5 ratio of polyunsaturated fatty acids omega-3 and omega-6 was most favorable for treating cardiovascular diseases (Klimakhin et al. 2015). Quality in oils is the ratio of unsaturated fatty acids to saturated fatty acids. The high rate of this ratio shows the quality of the oil and its importance in terms of benefiting human health. Fatty acid compositions determined in our study are compared with other oils (Table 3). According to Table 3, the highest ratio was in canola. The other highest unsaturated fatty acids rates are safflower, sunflower and olive, respectively. In this study, cocklebur oil was the second highest oil with unsaturated fatty acid ratio.

Table 2. Fatty acid compositions of cocklebur oil

Fatty acid	Structure	Formula	Amount (%)
Myristic	14:0	$C_{14}H_{28}O_2$	0.034
Palmitic	16:0	$C_{16}H_{32}O_2$	5.902
Palmitoleic	16:1	$C_{16}H_{30}O_2$	0.067
Stearic	18:0	$C_{18}H_{36}O_2$	2.697
Oleic	18:1	$C_{18}H_{34}O_2$	16.874
Linoleic	18:2	$C_{18}H_{32}O_2$	72.624
Linolenic	18:3	$C_{18}H_{30}O_2$	0.255
Arachidic	20:0	$C_{20}H_{40}O_2$	0.036
Gondoic	20:1	$C_{20}H_{38}O_2$	0.16
Behenic	22:0	$C_{22}H_{44}O_2$	0.84
∑SFA			9.509
∑MFA			17.101
∑PFA			72.879
Total			99.489

∑SFA: Saturated fatty acids, ∑MFA: Monounsaturated fatty acids, ∑PFA: Polyunsaturated fatty acids

Table 3. Fatty acid and (P/S) percentages of some crops (Baydar and Erbas 2014; Gursoy 2019)

Species	Unsaturated Fatty Acid (%)	Saturated Fatty Acid (%)	P / S Ratio
Sunflower	89	11	8.1
Soybean	85	15	5.7
Peanut	82	18	4.6
Olive	86	14	6.1
Canola	94	6	15.7
Safflower	90	10	9
Cocklebur*	90	9.5	9.5

*Our results

Macro- and microminerals content

Macro- and microminerals concentrations obtained our study are compared with other oil crops (Table 4). This study showed that the highest macromineral and micromineral contents are K and P and Na, respectively. The basic nutrients needed for the production of plants are also essential for the growth and development of all living things. Minerals, which constitute 4%–6% of the human body, are critical in nutrition (Klimakhin et al. 2015). The most abundant element was measured as Na at 0.15±0.02 ppm. Zn and Fe contents of cocklebur were found to be low compared to K and P levels. Mineral K in sunflower, sesame, flaxseed, soybean, corn and olive oil have detected in the range of 5.93-47.2 ppm. It is an important mineral in plants for metabolic, physiological and biochemical functions (Iskander 1993, İmer and Taşan 2018, Demir and Taşan 2019). Cocklebur seed detected the trace elements Se, Mn, Zn, and Cu, which are valuable for humans (Klimakhin et al. 2015).

Table 4. Macro and micro minerals in the vegetable oils (ppm) (Imer and Taşan 2018).

Vegetable oil	K	P	Fe	Mn	Zn	Cu	Na
Sunflower	7.10±1.43	22.39±4.41	1.03±0.20	**	0.58±0.10	**	4.45±0.32
Safflower	1.99±0.29	5.96±0.57	0.74±1.10	**	0.35±0.05	**	6.97±0.90
Flaxseed	4.40±0.49	17.43±2.35	0.86±0.14	**	0.39±0.06	**	9.73±1.18
Hazelnut	9.69±1.39	36.49±3.08	0.45±0.06	**	0.32±0.07	**	7.93±1.41
Sesame	16.26±3.79	50.10±10.78	2.16±0.57	**	0.24±0.07	**	8.69±1.92
Peanut	51.73±13.92	36.57±7.71	1.46±0.26	**	0.14±0.04	**	8.23±1.09
Cocklebur*	17.53± 2.13	173.00±0.11	0.11±0.02	**	0.02±0.01	**	0.15±0.02

Values are mean±SD in each group. * Our results, **not detected

Oxidative stress parameters and AChE activities of samples

Results of antioxidant enzyme activities of erythrocytes and leucocytes

Antioxidant enzyme activities (GST, SOD, GPx and CAT) of 0.01, 0.02, 0.04 µL mL^{-1} oil which was obtained from the seeds of *X. strumarium*, treated human leucocytes and erythrocytes did not show any differences compared to control group (Table 5 and Table 6). Treatment with higher doses of *X. strumarium* oil significantly decreased cell antioxidant enzyme activities.

Table 5. Enzyme activities of different treatment groups of erythrocytes

Groups (µL mL^{-1})	SOD (U $mgHb^{-1}$)	CAT (U $mgHb^{-1}$)	GPx (U $mgHb^{-1}$)	GST (U $mgHb^{-1}$)	AChE (U $mgHb^{-1}$)
Control	596.94 ±18.21a	372.18±9.94a	69.62±6.76a	41.23±2.09a	29.1±4.57a
0.01	588.76±9.9 a	374.51±11.25a	68.37±7.11a	39.03±3.41a	28.78±2.81a
0.02	579.86±11.53a	351.7±15.02a	65.92±5.78a	39.28±2.67a	26.19±3.22a
0.04	571.64±18.47a	349.76±16.82a	61.45±5.99a	36.42±4.02a	26.66±3.73a
0.08	411.58±7.22b	243.54 ± 8.22b	59.01±8.17a	28.44±3.29b	25.41±2.94a
0.16	342.06±8.15c	238.71±16.11b	35.06±3.92b	20.08±2.24c	19.71±2.62b
0.32	280.71±10.03d	152.02±9.88c	24.04±4.18c	15.19±2.15d	18.09±1.1b
0.50	216.08±6.99e	101.29±12.04d	13.12±5.03d	11.17±3.65d	14.28±2.04c
1.00	127.91±12.56f	62.49±8.67e	5.11±2.12e	2.26±0.69e	10.16±1.88d

Values are mean ± SD in each group. Significance at $P < 0.05$. Within each column, means superscript with different letters are significantly different.

Table 6. Enzyme activities of different treatment groups of leucocytes

Groups (µL mL^{-1})	SOD (U $mgprotein^{-1}$)	CAT (U $mgprotein^{-1}$)	GPx (U $mgprotein^{-1}$)	GST (U $mgprotein^{-1}$)
Control	467.88±23.27a	301.92±17.01a	64.12±7.65a	34.22±5.21a
0.01	455.1±16.24a	292.31±14.47a	63.83±6.17a	33.06±7.21a
0.02	449.53±16.8a	293.05±11.73a	60.23±5.29a	30.48±3.76a
0.04	438.11±14.32a	284.08±16.72a	57.8±3.01a	30.63±4.89a
0.08	359.34±18.05b	216.92 ± 8.83b	48.06±5.14b	26.43±8.91a
0.16	313.7±11.14c	188.27±10.11c	37.77±4.36c	15.52±2.73b
0.32	234.31±13.22d	140.47±7.98d	21.01±7.82d	8.67±3.01c
0.50	173.06±21.74e	98.52±17.3e	18.64±5.24d	6.89±2.72c
1.00	99.52±27.24f	46.15±22.05f	4.47±2.09e	2.13±1.01d

Values are mean±SD in each group. Significance at $P < 0.05$. Within each column, means superscript with different letters are significantly different.

Results of lipid peroxidation levels of erythrocytes and leucocytes

MDA levels (GST, SOD, GPx and CAT) of 0.01, 0.02, 0.04 µL mL^{-1} *X. strumarium* seed oil treated human leucocytes and erythrocytes did not show any differences compared to control group (Figure 1 and Figure 2). Treatment with higher doses of *X. strumarium* oil significantly elevated cell MDA levels.

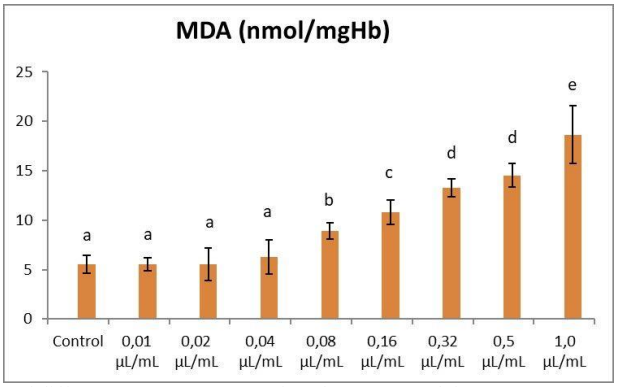

Figure 1. MDA levels of different treatment groups of erythrocytes. Each bar represents mean ± SD in each group. Columns superscripts with different letters are significantly different. Significance at $P < 0.05$.

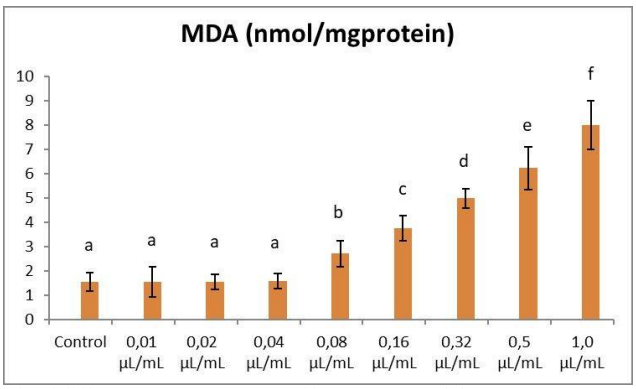

Figure 2. MDA levels of different treatment groups of leucocytes. Each bar represents mean±SD in each group. Columns superscripts with different letters are significantly different. Significance at $P < 0.05$.

Results of AChE activities of erythrocytes

Lower doses of *X. strumarium* seed oil treated erythrocytes did not show significant changes compared to control (Table 5). Treatment with higher doses (above 0.04 µL ml^{-1}) showed decreased AChE activity as compared to control cells.

Results of mitotic index

MI values are summarized in Figure 3. There was a decrease in MI values with increasing doses of *X. strumarium*. Significant changes were observed in 0.16, 0.32 and 0.50 µL mL^{-1} concentrations compared with the control ($P < 0.05$).

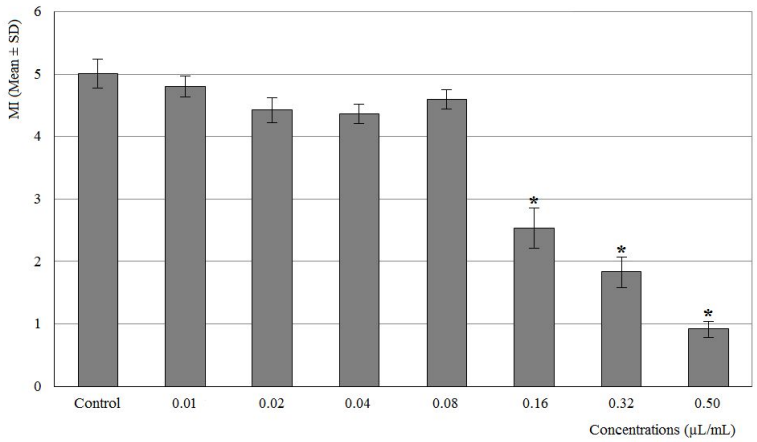

Figure 3. Mitotic index values in peripheral lymphocyte cultures exposed to different concentrations of *X. strumarium*. *Significantly different compared to control ($P < 0.05$).

Results of replication index

RI values are shown in Table 7. There was a decrease in RI values with increasing concentrations. 0.16, 0.32 and 0.50 µL mL^{-1} concentrations compared with the control ($P < 0.05$).

Table 7. Replication index values in peripheral lymphocyte cultures exposed to different concentrations of *X. strumarium.*

Concentrations (µL mL^{-1})	RI (Mean ± SD)
Control	1.282 ± 0.026
0.01	1.212 ± 0.019
0.02	1.174 ± 0.013
0.04	1.170 ± 0.011
0.08	1.184 ± 0.015
0.16	1.080 ± 0.010*
0.32	1.056 ± 0.006*
0.50	1.039 ± 0.008*

*Significantly different compared to the control ($P < 0.05$)

Results of micronucleus

MN rates are summarized in Figure 4. There was an increase in MN values with increasing doses of *X. strumarium*. Significant changes were observed in 0.16, 0.32 and 0.50 µL mL^{-1} concentrations compared with the control ($P < 0.05$).

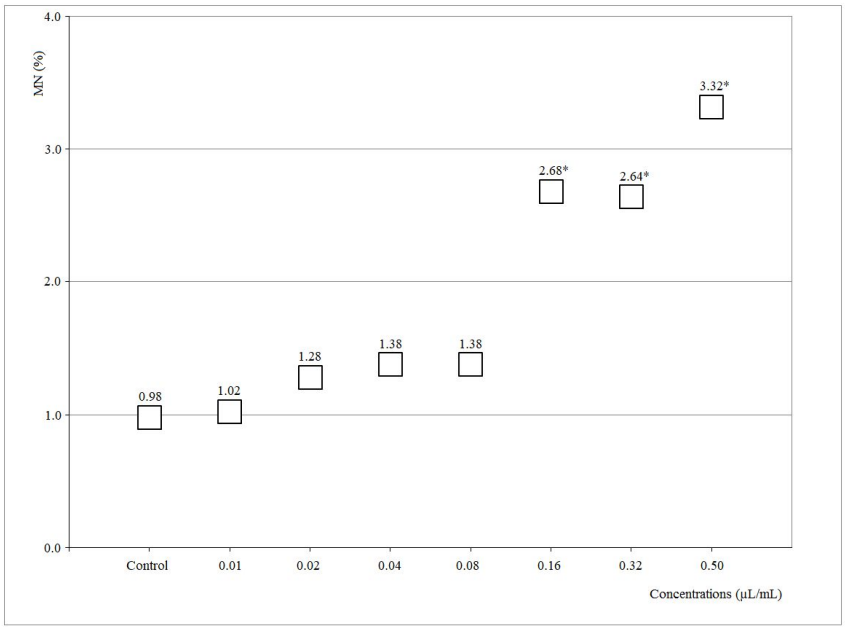

Figure 4. Micronucleus values in peripheral lymphocyte cultures exposed to different concentrations of *X. strumarium*. *Significantly different compared to control ($P < 0.05$).

Cocklebur has been used in many areas from traditional medical treatment to modern pharmacology and, for this reason, it is very important to determine effects on the human genome. The genotoxicity results showed a genotoxic effect to the dose-dependent (0.16, 0.32, and 0.50 µL mL^{-1}). In addition, MI and RI provide a measure of proliferative capacity of the cells and therefore can be used as indicators of the cytotoxic effects of the extracts investigated. The cytotoxicity results showed significant decreases in MI and RI values (0.16, 0.32, and 0.50 µL mL^{-1}). Witte et al. (1990) indicated that *X. strumarium* had medium to strong allergenic effects and was poisonous to mammals, and carboxyatractyloside and atractyloside were considered to be the basic toxic compounds. In literature, some reports have recorded these toxic and allergenic effects of monomers or extracts of *X. strumarium*; however, they are limited with few in vitro and usually in vivo animal experiments. A review summarized the toxicities and side effects of extracts of *X. strumarium*. These effects are death, weight loss, enlarged hepatic cell space, karyolysis, inflammatory cell infiltration, depressing the action of central nervous system, and decreasing hatch rate in mice, swine and zebrafish (Fan et al. 2019). Xue et al. (2014) showed that carbxyatractyloside and atractyloside inhibited cell proliferation and improved LDH activity at high concentration (100 µmol L^{-1}). In addition, Yu et al. (2013) reported that the water extracts of *X. strumarium* fruits could inhibit growth of HK-2 cells at high concentrations (100 µg mL^{-1}). Moreover, hydroalcoholic extracts of aerial parts of *X. strumarium* also caused DNA damage at different concentrations (25-100 µg mL^{-1}) through comet assay, chromosome aberrations, and sister chromatid exchanges (Piloto 2014). Su et al. (2016) indicated that the different components of *X. strumarium* had cytotoxic potentials and the ethyl-acetate fraction of methanol extracts of fruits of *X. strumarium* was the most toxic part. Despite all these reports, there is no study showing the cytotoxic and genotoxic effects of the cocklebur on human peripheral lymphocytes in vitro.

The toxic effect of oils obtained from plants has also been investigated in previous studies (Zaoui 2002). Exposure to xenobiotics such as plant oils may cause lipid peroxidation (LPO) and change the antioxidant enzyme activities such as CAT, SOD, GST and GPx. These enzymes are parts of first line defence against reactive oxygene species (ROS) (Zaoui 2002, Bas and Kalender 2016). These parameters are proven indicators of oxidative stress process (Pathak and Khandelwal 2006). Because they are potential targets for xenobiotic toxicity (Patra et al. 2011). So, we investigated their activities for understanding the effects of *X. strumarium*'s seed oil. In our study, SOD, CAT, GST and GPx activities of human erythrocytes and leucocytes significantly decreased in increasing doses of oil compared with not-treated cells, especially from the 0,08 µL mL^{-1}. As shown in numerous studies, changing of antioxidant enzyme activities correlate with increased concentrations of malondialdehyde (MDA) (Bas and Kalender 2016). MDA is the main oxidation product of LPO. Therefore, increasing in the MDA level is a major indicator of LPO (Comelekoglu et al. 2012). For this reason, we investigated its value for assignation of oxidative stress caused by LPO in leucocytes and erythrocytes. LPO has a main function in xenobiotic toxicity because of causing unwanted effects on cell and organelle membrane rigidity and osmotic fragility (Bas et al. 2014). In this study, increased MDA levels were observed in *X. strumarium*'s seed oil treated blood cells. This increase may explain with harmful effects of *X. strumarium*'s seed oil on cell membranes.

Acetylcholinesterase (AChE) has a main role in neurotransmission mediated by acetylcholine which is a neurotransmitter. The evaluation of AChE activity in cells is an important mark that diagnosis of poisoning induced by reversible and irreversible inhibitors (Santi et al. 2011). Some components of essential oils can cause neurotoxic effects on organisms, especially monoterpenes that found in plant oil can be act as competitive inhibitor of AChE (Kostyukovsky et al. 2002). In a previous study, essential oil of *Hypericum perforatum* caused decreasing of AChE activity (Bas and Ersoy2020). Also, Polatoğlu et al. (2016) indicated that AChE enzyme activity may be decreased by plant oil exposing. According to the results of MDA values, activities of AChE and antioxidant enzymes, it is clear that *X. strumarium*'s seed oil causes adverse effects in human leucocytes and erythrocytes especially from the 0.08 µL mL^{-1}.

Conclusion

The fatty acid component is changeable to a large scale depending on many of factors. Therefore, to know the variation occurred in fatty acid component of an oil-seed under given condition is very important for oil quality. Oil quality depends on food technology and processing manner for crude oil. Knowing the fatty acid component of vegetable oil makes it possible to produce oil for special using purposes. For this purpose, it would be possible to produce suitable oils by cultivating the desired species in suitable conditions. In edible oils, oleic and linoleic acid are the most important fatty acid components that are determine the oil quality. These two fatty acids were found to be significant in cocklebur seed oil. According to the results, it has been determined that it is close to the edible oils. However, in this study, it is thought that the chemical components found in cocklebur oil, which are different from edible oil, have toxic effects. In the genotoxic study, peripheral blood culture was performed in a wide dose range. Although cytotoxic genotoxic effects are detected in high concentrations, cocklebur oil can be evaluated at lower doses. Antioxidant results also support this opinion. Thus, it is thought that cocklebur seed oil, known as weed, cannot be recommended as alternative edible oil, but it can be used in different areas as an industrial crop and will contribute to the economically. It was concluded that more studies should be done on this vegetable oil.

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Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

The contribution of the authors is equal

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