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Scientific Research

Study of subchronic toxicity of camelina oil and its effect on biochemical factors and hematological parameters

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ABSTRACT

Camelina oil contains large amounts of unsaturated fatty acids and phenolic compounds, which affect the amount of blood factors such as blood lipids. The presence of these compounds reduces the deposition of fat in the veins and reduces the mutagenicity and carcinogenicity of factors such as benzopyrene. The aim of this research is to evaluate the safety and effect of camellia oil as an edible oil on the growth, tissue and blood factors of Wistar rats in order to investigate its use in human nutrition. During this period (90 days), 40 male Wistar rats in 4 groups were administered Camelina oil with doses of 0.1, 1 and 10 ml daily compared to the control group, in blood and biochemical parameters such as lipid factors. Blood, ALT, AST, ALP and white and red blood cells were evaluated. In ALT, Cr, LDL, total cholesterol, WBC, PDW and RBC factors, no significant difference was observed between the groups and the control group. But the reduction of urea, TG, AST, RDW, MCHC occurred in different groups compared to the control group, and this reduction was associated with a significant difference. A significant difference was observed in blood glucose level in two groups with 1 and 10 ml diet. Also, the exposure of this oil to the studied doses did not cause any pathological and clinical effects in the studied animals compared to the control group animals in a period of three months. Also, the results of this study showed that due to the high amount of unsaturated fatty acids (linolenic acid and linoleic acid), tocopherol and other antioxidants, camellia oil can be effective in increasing the immunity of the cellular level of the body and human health.

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1. Introduction

Considering today's country's need for edible oils, it is of great importance to identify plants containing suitable fatty acid compounds that have the ability to grow in the country's climatic conditions. Therefore, Camelina oil, which is one of the new vegetable oils, is of special importance due to its special agricultural conditions and its high adaptability in different agricultural and climatic conditions in the country [1]. Camelina (Camelina Sativa) is an oily-medicinal plant and belongs to the family of Brassicaceae, which is namedFalse flax, wild flax, German sesame andgoldofpleasure Is known. In addition to food consumption, it is also used in the production of biofuel [2]. This plant contains high unsaturated fatty acids, polyphenols, vitaminsAND and carotene. About 30 to 40% of the dry weight of camellia seeds are oil compounds, which are 64% polyunsaturated fatty acids, 30% monounsaturated fatty acids, and 6% saturated fatty acids [3]. Camelina oil contains high amounts of fatty acids arachidic acid, linoleic acid, alpha linolenic acid, eicosa dienoic acid and omega 6 fatty acids and a high amount of tocopherol compounds containing bioactive compounds such as saponintea, flavonoids, terpenoids and polyPhenolis Modern pharmacological research has shown is that these compounds can reduce the content of cholesterol, triglycerides,LDL[1] and be effective in protecting the heart and preventing cancer [4]. Also, saturated fatty acids in the diet were found to be an important determining factor in increasing the levelLDL Is. Therefore, this category of fatty acids has been directly related to cardiovascular risk for a long time (similar effect on cholesterol levels andHDL[2] usually ignored). In this case, the debate is still ongoing, but the consensus of opinion is that the consumption of saturated fat should be limited [5]. In oils, in addition to phenolic compounds and saturated and unsaturated fatty acids, there are also other compounds such as polycyclic aromatic hydrocarbons. Some of thesePAHs[3] which are known as mutagenic and carcinogenic compounds are formed in foods from carbohydrates at high temperatures in the absence of oxygen or from amino acids and fatty acids (such as benzopyrene). However, the production of these compounds at temperatures of 100 to 150 degrees Celsius has also been reported [6]. Also pollution toPAHsIn edible oils, it occurs during the drying process of oil seeds or contamination during the solvent extraction process [7]. Studies show that the presence of antioxidants and phenolic compounds can determine the amountPAHs It is effective in heated products. On the other hand, the presence of essential fatty acids in this oil, in addition to the body's need for these fatty acids, can reduce the amount of triglycerides in the blood and reduce cardiovascular diseases in the society [8,9]. In this study, due to the richness of camelina oil in antioxidant compounds and essential fatty acids, the aim of investigating the effect of these compounds on reducingPAHs and the amount of blood factors in living organisms so that a solution can be found to reduce benzopyrene in food by using appropriate formulations.

2- Materials and methods

2-1- The strain used in the research

In this research, the indigenous species of Camelina seeds DH1025 was used.

2-2-Study population

In this study, 40 Wistar rats were used in 4 groups to evaluate the subchronic toxicity of Camelina oil.

2-3-Keeping and preparing animals

40 adult male Wistar rats with an average weight of (220±22) grams were randomly divided into 5 groups of ten. These animals were purchased from the Laboratory Animal Department of Pasteur Institute. The maintenance method was carried out according to the method of Ahmed et al.10].

Table 1 Doses used to treat animals

Studied groups	name
1	control (normal saline)

[1]. Low-density lipoprotein

[2]. high-density lipoprotein

[3]. Ploycyclic Aromatic Hydrocarbons

2	Treatment of animals with a dose of 0.1 ml of camellia oil in the diet
3	Treatment of animals with a dose of 1 ml of camellia oil in the diet
4	Treatment of animals with a dose of 10 ml of camellia oil in the diet

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2-4- The method of preparing poisons and determining the dose

To determine the doses used in this study, OECD standard protocol No. 408 entitled Subchronic Oral Toxicity-Fixed Dose Method was used.

2-5- The stage of taking blood from animals

After the end of the elapsed time (90 days), the animals were anesthetized by ether and finally, blood was taken from the hearts of the animals. First, the head of the syringe was passed on the left side of the chest between the fifth and sixth ribs and pushed forward towards the heart and blood collection was performed. Blood samples were centrifuged at 3000 rpm for 20 minutes and their serum was separated for biochemical tests.

6-2- Measurement of hematology markers

Hematology factors were performed using a Siemens cell counter (ADVIA 120, Hematology system) made in Germany. Hematological markers[4]WBC،RBC[5]، PLT[6],GLUE[7]، MCHC[8] and MCH,Mcv[9] And[10] Hbg was measured**[**11**].**

7-2- Measurement of biochemical markers

Biochemical markers were performed using autoanalyzer model (BT3000) made in Italy and kit used by Biosystem company made in Spain. Blood samples were centrifuged at 3000 rpm for 20 minutes and separated to measure biochemical markers. Biochemical factors such as[11]AST،[12]ALP،[13]Cr[14] BUN was investigated**[**11**].**

8-2-stage of dismembering the animal

After taking blood from the animal, in order to observe pathological lesions from different organs such as heart, lung, kidney, liver, the tissue was completely or partially removed and placed in containers containing 10% formalin buffer to be sent to the pathology laboratory. The sent containers have a specification label and also contain information about the poison used and explanations about the place of tissue collection, and to make the histology tests easier, a number was assigned to each of the prepared samples, which indicated the type and characteristics of the tissue. 10% buffered formalin was used to fix and stabilize tissues. The samples were placed in formalin for 14 days.

9-2-Pathology tests

At this stage, the desired samples were placed in tissue processor containers with different alcohol percentages according to the standard protocol. Then, the samples were placed in melted paraffin, this substance penetrated into the tissue and penetrated into the cracks and joints of the tissue, and at the temperature of the tissue laboratory, the tissue became rigid and could be cut with a microtome machine. In the next step, the molded samples were cut with a thickness of 5 microns using a microtome, and the samples were stained on the slide. A microscope (Labomed Lx 400 USA) was used to observe and analyze the slides.

2-10-how to perform statistical methods

Data obtained from each group using statistical software*SPSS*and were subjected to statistical analysis using ANOVA-ONE WAY test. The significant level of difference between samples ($P<0.05$) was considered and the obtained results were placed in separate tables for analysis and comparison.

3-Results

3-1-Clinical symptoms

[4]. white blood cells

[5]. Red Blood Cell Count

[6]. Platelet

[7]. lymphocytes

[8]. Mean Corpuscular Hemoglobin

[9]. Mean Corpuscular Volume

[10]. Hemoglobin

[11]. Aspartate Amino transferase

[12]. Alkaline Phosphatase

[13]. Creatinine

[14]. Blood Urea Nitrogen

The results of clinical symptoms showed that after oral administration of Camelina oil with doses of 0.1, 1 and 10 ml of Camelina oil for three months, there were no unusual clinical symptoms such as neurological symptoms, digestive disorders, respiratory complications, skin toxicity, etc. in the animals. The study was not observed in the exposure groups compared to the control group.

2-3-weight changes

After oral administration of camelina oil with doses of 0.1, 1 and 10 ml for three months in rats, significant changes in weight were observed in the studied animals compared to the control group, and the weight of the studied animals increased in the studied groups. The comparison with the control group was shown (Table 3).

Table 2 Weight changes in low, medium and high dose groups fed with camellia oil.

Final weight	initial weight	Studied groups
364±32 [b]	202±20 [a]	Control
342±35 [a]	200±22 [a]	First
372±34 [c]	204±22 [a]	Second
388±37 [d]	201±24 [a]	Third

*Different lower-case letter indicate in significant difference $p<0.05$

3-3-Biochemical parameters

Oral exposure of studied animals to doses of 0.1, 1 and 10 ml of camellia oil for three months, significant changes in biochemical parameters, alanine aminotransferase enzyme (LTA), creatinine (Cr), low-density lipoprotein (LDL) and total cholesterol (Cholestrol) were not significantly different compared to the control group (Table 4).

Table 3 Biochemical parameters in low, medium and high dose groups fed with camellia oil.

	Control	First	Second	Third
Glucose) mg/dL(	87.85 ± 9.33[a]	87.85 ± 8.25[a]	106.5 ± 5.74[b]	106.12 ± 5.01[b]
Urea) mg/dL(	35.5 ± 4[b]	32.3 ± 2.98 [ab]	29.33 ± 2[a]	30 ± 2.06 [a]
Cr) mg/dL(	0.68 ± 0.1 [a]	0.67 ± 0.07 [a]	0.68 ± 0.06[a]	0.65 ± 0.05[a]
ALL) U/L(	52.28 ± 5.22 [a]	56.12 ± 7.79[a]	58.75 ± 5.7[a]	59.8 ± 9.39[a]
AST) U/L(	134.11 ± 11.12[b]	116.33 ± 6.5[ab]	88 ± 13.72 [a]	100 ± 14.84[ab]
CHO) mg/dL(	56.37 ± 5.34 [a]	69.78 ± 5.56 [a]	72.4 ± 10.61 [a]	64 ± 5.58 [a]
HDL	19.1 ± 2.81 [a]	18.7 ± 1.83 [a]	27.55 ± 4.03 [b]	17.6 ± 2.12 [a]
LDL	13.6 ± 1.58 [a]	13.4 ± 1.17 [a]	12.2 ± 1.13 [a]	12.4 ± 1.07 [a]
TG	35.5 ± 5.42 [b]	29.4 ± 4.53 [a]	34.4 ± 4.22 [ab]	30 ± 5.89 [a]

Control = First, low dose = Second, medium dose = Third, high dose.
urea, creatinine (Cr), alanine aminotransferase (LTA), aspartate aminotransferase (AST), cholesterol (CHO), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride(TG). *Significant differences are shown in lowercase letters.

In high-density lipoprotein (HDL), the amount of this compound in the average dose was significantly different from other groups and the control group. The amount of blood triglyceride factor has also decreased in the groups compared to the control group. A decrease in the amount of aspartate aminotransferase (AST), blood urea (urea), and triglyceride (TG) was observed compared to the control group, which according to Table 4 had the largest difference with the medium dose group and a significant difference between the control group and the group A moderate dose was observed. It also showed that the blood glucose level in the animals made significant changes in the average and higher limits.

3-4-hematological factors

Oral exposure of the studied animals to doses of 0.1, 1 and 10 ml of camellia oil for three months, no significant changes in hematological factors such as white blood cells (WBC), red blood cells (RBC), hematocrit (HCT) and hemoglobin HGB, platelet count (PLT), procalcitonin (PCT), mean platelet size (MPV) and platelet diameter (PDW) compared to the control group. Also, there was no decrease in red blood cell diameter (RDW),

average red blood cell hemoglobin concentration (MCHC), hemoglobin weight in one red blood cell (MCH) and average red blood cell volume (Mcv) in the low and medium dose groups compared to the control group. In the high dose group, the amount of these factors is higher. Also, according to the normal concentration of these factors in rats, these changes are within the normal range (Table 5).

Table 4 Changes in hematological parameters in low, medium and high dose groups fed with camellia oil.

	Control	First	Second	Third
WBC (10^3/mm)	5.8 ± 2.4[a]	7.35 ± 1.7[a]	8.46 ± 2.12[a]	7.29 ± 1.7[a]
RBC·(10^6 /µl(	7.22 ± 1[a]	8.04 ± 0.46[a]	8.02 ± 0.4[a]	8 ± 0.34[a]
HGB) g/dL(	13.46 ± 1.61[a]	14.26 ± 0.67[a]	13.95 ± 0.63[a]	14.99 ± 0.66[a]
HCT %)(	38.86 ± 4.96[a]	41.64 ± 2.52[a]	40.87 ± 1.86[a]	42.33 ± 1.46[a]
MCV(fl)	53.91 ± 1.44[c]	51.80 ± 1.54[ab]	51.10 ± 1.36[a]	52.93 ± 1.11[bc]
MCH(pg)	18.7 ± 0.71[b]	17.75 ± 0.71[a]	17.43 ± 0.69[a]	18.75 ± 0.6[b]
MCHC(g/dL)	34.69 ± 0.62[ab]	34.28 ± 0.82[a]	34.14 ± 0.6[a]	35.41 ± 0.5[b]
PLT) 10^3/mL(	649.143 ± 121.9[a]	570.75 ± 127.59[a]	563.28 ± 145.38[a]	584 ± 88.28[a]
DMV	13.47 ± 0.83[b]	12.23 ± 0.44[a]	12.33 ± 0.44[a]	13.32 ± 0.78[b]
PCT	0.15 ± 0.09[a]	0.15 ± 0.09[a]	0.15 ± 0.08[a]	0.17 ± 0.06[a]
MPV	3.84 ± 1.18[a]	3.23 ± 0.64[a]	3.77 ± 1.1[a]	3.38 ± 0.88[a]
PDW	16.13 ± 0.95[a]	16.05 ± 0.74[a]	15.78 ± 0.95[a]	15.83 ± 0.86[a]

Control = First, low dose = Second, medium dose = Third, high dose.

White blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean red blood cell volume (Mcv), weight of hemoglobin in a red blood cell (MCH)), mean hemoglobin concentration of red blood cells (MCHC)), platelet count (PLT), red blood cell diameter (RDW), procalcitonin (PCT), mean platelet size (MPV) and platelet diameter (PDW). *Significant differences are shown in lowercase letters.

5-3- Histopathological evaluation changes

Oral exposure of the studied animals to doses of 0.1, 1 and 10 ml of camellia oil in the diet for three months, no significant pathological complications such as bleeding, necrosis, hyperemia, hyperplasia, degeneration, inflammation, etc. in the investigated tissues. did not develop compared to the control group (Figure 1).

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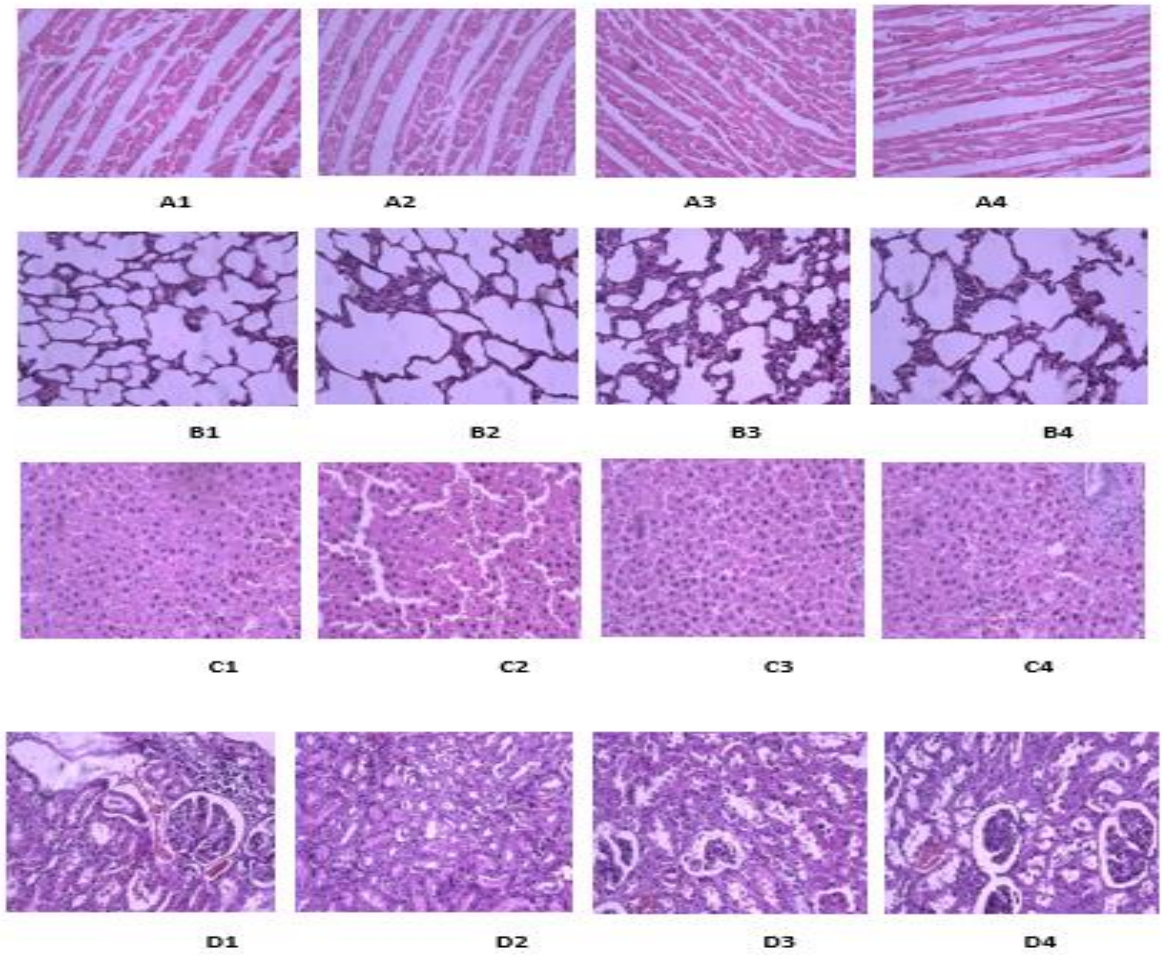

Fig 1 Normal heart tissue in animals, respectively, control, low dose, medium dose and high dose groups (A1,A2,A3,A4).
Normal lung tissue in animals, respectively, control groups, low dose, medium dose and high dose (B1, B2, B3, B4).
Normal liver tissue in animals, respectively, control groups, low dose, medium dose and high dose (C1, C2, C3, C4).
Normal kidney tissue in animals, respectively, control groups, low dose, medium dose and high dose (D1, D2, D3, D4).(40x magnification).

4- Discussion and conclusion

Many researchers and public health organizations confirm the undeniable connection between the use of oils containing essential fatty acids, especially linoleic fatty acid, and lower incidence of cardiovascular diseases as well as neurodegeneration and cancer. In addition to the effects of unsaturated and essential fatty acids, the reduction of coronary artery risks should be attributed to the polyphenols present in these oils [12]. By examining the clinical symptoms after oral administration of Camelina oil with doses of 0.1, 1 and 10 ml for three months, no abnormal clinical symptoms were observed compared to the control group, and in addition, in terms of weight, after oral administration of Camelina oil with doses of 1 0.1 and 10 ml for three months, a significant increase in weight was observed in the investigated animals compared to the control group. Based on the research done by Chang et al. on the effect of camelina oil on the weight and blood factors of mice, they concluded that the diet containing camelina oil showed an increase in weight in mice, and also camelina oil affects the lipid characteristics of mouse blood serum. The amount of surface[15]TC decreased significantly. These results showed that camellia oil supplement with moderate fat intake can be

[15]. Total cholesterol

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effective on health, body weight control and blood lipid factors [13]. Camelina oil can also enhance the metabolism of high-density lipoprotein (HDL) to shrink HDL particles, and it has lipid-lowering activity by regulating blood lipid metabolism and protecting liver function [14]. In general, it can be said that the oral exposure of the studied animals in the high-dose camelina oil group for three months caused a non-significant increase in white blood cells and a significant difference in red blood cells, which can be attributed to the beneficial effects of oleic acid. And linoleic acid and other essential fatty acids affect the amount of blood factors, attributed to other minor components with antioxidant and anti-inflammatory properties found in this oil [15]. A decrease in the amount of aspartate aminotransferase (AST), blood urea (urea), compared to the control group, was observed, which was in accordance with the studies of Afri Mansaw and colleagues. In the review, the researchers investigated the addition of camelina and chia oil in the diet of Sparusaurata, L. fish and concluded that although the inclusion of camelina and chia oil changed the fatty acid composition of the experimental diets, slight differences in the digestibility of fatty acids and also reduced showed triglyceride and cholesterol levels, while glucose levels were not affected. Addition of camelina or chia oils to the diets of these fish increased the genes responsible for fatty acid synthesis, lipolyslipids and lipogenesis, which enhanced fat deposition. This resulted in histological changes, characterized by the accumulation of fat droplets in the fish intestine. Given that lipid droplets are intracellular organelles that store neutral lipids for use as an energy source in membrane synthesis and lipid production and accumulate when ingested lipids are not oxidized. Accumulation of lipids led to the displacement of nuclei and cytoplasmic organelles in the intestine of fish fed diets in the study [16]. In addition to the reducing effects in blood factors, anti-arrhythmic and antioxidant effects of oil seeds and long-chain unsaturated fatty acids such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) on triglycerides, platelet function, blood pressure and on less production of adhesion and proteins. showed pro-inflammatory by the artery wall [17, 18], and the effect of consuming oils containing EPA and DHA at the rate of 500 ml twice a week for the prevention of cardiovascular disease has been proven. Adequate intake of omega-3 and omega-6 in the diet through oilseeds (safflower, canola, and soybean and walnut oils), namely alpha-linolenic acid (ALA; 18:3:3), significantly reduces the risk of coronary artery disease and sudden death. especially in elderly people[19,20,21]. On the other hand, the reduction of LDL in the blood is also in accordance with the study conducted by Karonen in 2002. Based on the research done by Karvonen et al., who compared the effects of camellia oil on blood fat and its fatty acid composition with rapeseed oil and olive oil. Alpha-linolenic acid in the blood lipids of people who consumed camelina oil was reported to be 2.5 times higher than those who consumed rapeseed oil and 4 times higher than those who consumed olive oil. Also, 2 metabolites of alpha-linolenic acid (eicosapentaenoic acid and docosapentanoic acid) were significantly higher in people who consumed camelina oil. The level of LDL in the camelina oil group decreased by 12.2%, the rapeseed oil group by 5.4%, and the olive oil group by 7.7%. As a result, Camelina oil had a greater effect on LDL reduction compared to rapeseed oil and olive oil [22]. The results of this research showed that Camelina oil, due to the high amount of unsaturated fatty acids, especially linolenic acid and linoleic acid, tocopherol and other antioxidants, can be effective in increasing the immunity of the cellular level of the body and human health, and considering the adaptability of this oil plant to the conditions Due to different climates and low water requirements and resistance to diseases and pests, it is important to evaluate the safety of camellia oil as an edible oil. Also, the results of this research showed that after oral administration of Camelina oil with doses of 0.1, 1 and 10 ml of Camelina oil for three months, there was a significant decrease in blood factors such as TG, blood glucose, urea, AST, MCV, MCHC. . Of course, it is necessary to carry out this study in food rations with different doses and more samples.

5- Resources

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مقاله علمی_پژوهشی

مطالعه سمیت تحت مزمن روغن کاملینا و تاثیر آن بر فاکتورهای بیوشیمیایی و پارامترهای خون شناسی

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چکیده

روغن کاملینا حاوی مقادیر زیادی اسیدهای چرب غیر اشباع و ترکیبات فنولی است که این ترکیبات بر میزان فاکتورهای خونی نظیر چربی خون تاثیر دارند. وجود این ترکیبات باعث کاهش رسوب چربی در رگها و کاهش جهشزایی و سرطانزایی عواملی مانند بنزوپیرن میشود. هدف از این پژوهش ارزیابی ایمنی و تاثیر روغن کاملینا به عنوان روغن خوراکی در رشد، بافت و فاکتورهای خونی رت‌های نژاد ویستار به منظور امکان بررسی استفاده آن در تغذیه انسان است. در طی این دوره (۹۰ روز) تعداد ٤۰ سر موش ویستار نر در ٤ گروه تحت تجویز روغن کاملینا با دوزهای ۰/۱، ۱ و ۱۰ میلی‌لیتر به صورت روزانه در مقایسه با گروه کنترل، در پارامترهای خونی و بیوشیمیایی نظیر فاکتورهای چربی خون، آنزیم ALT، AST،ALP و گلبول های سفید و قرمز خونمورد ارزیابی قرار گرفت.در فاکتورهای ALT، Cr، LDL، میزان کلسترول کل، WBC ، PDW و RBC تفاوت معنی داری بین گروهها وگروه کنترل مشاهده نشد.اما کاهش میزان اوره، TG، AST ،RDW، MCHC درگروههای مختلف در مقایسه با گروه کنترل اتفاق افتاد و این میزان کاهش با تفاوت معنی داری همراه بود. میزان گلوکز خون در دو گروه با جیره غذایی۱و۱۰ میلی لیتر تفاوت معنی دار مشاهده گردید. همچنین مواجهه این روغن با دوزهای مورد مطالعه هیچگونه عوارض پاتولوژیک و بالینی در حیوانات مورد مطالعه در مقایسه با حیوانات گروه کنترل در مدت زمان سه ماهه ایجاد نکرد. همچنین نتایج این مطالعه نشان دادروغنکاملینا با توجه به مقدار بالای اسیدچرب غیراشباع (اسید لینولنیک و اسید لینولئیک)، توکوفرول و سایر آنتیاکسیدان‌ها میتواند در افزایش ایمنی سطح سلولی بدن و سلامتی انسان موثر باشد.