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The Effect of Vegetable Oils on some Physiological Traits in Adult Male Rats

ABSTRACT

The study was conducted in the animal house at the Faculty of Veterinary Medicine/ Tikrit University to investigate the effect of different concentrations of four types obtained from Al-Emad oil production company, which is grape seed oil, pumpkin oil, black seed oil and olive oil, with two types of commercial oils obtained from local markets, namely sunflower oil and corn oil, on some physiological characteristics of adult male rats with a number of 28 animal which had been divided in to 7 Groups , each one contained 4 animals. Animals underwent a lightening cycle which has been divided into 12 light hour and 12 dark hour, with a fixed temperature at 25± 2 º C. Animals had been left for one week to be adapted with the new conditions and to be sure of diseases privation. Food and water had been given continuously by oral dosing with sufficient quantities along the period of the process. The results have shown a significant increase in the gained weight of laboratory animals. There is also an increase in the total number of red blood cells, hemoglobin, granulosa cells, lymphocytes and platelets when adding grape seed oil, nigella oil, pumpkin oil and olive oil. And a decrease in the number of white blood cells compared to the addition of sunflower oil and corn oil. There is also an increase in the values of immunoglobulins IgA, IgM, IgE and IgG When adding grape seed oil, pumpkin seed oil, black seed oil and olive oil and a decrease in the values of the liver enzymes (ALT) and (AST) compared to the addition of sunflower oil and corn oil.

INTRODUCTION

The plant kingdom includes many plant species that are a source of many natural products, The continuous emergence of drug-resistant bacteria poses a global threat to human health, and the discovery of alternative treatments is necessary, as bacterial resistance to antibiotics has reached an alarming stage, especially in developing countries (Williams *et al.*, 2018; Amann *et al.*, 2019).

Vegetable oils contain certain stable and effective components such as triglycerides, free fatty acids, phospholipids, tocopherols, sterols, waxes, squalene and others (Esmael *et al.*, 2020).

Vegetable oils are also rich in many biologically active metabolites such as alkaloids, tannins, terpenoids, saponins, flavonoids and phenolic compounds, which can produce a physiological effect on the human body (Shakya, 2016). As such affects largely immune enzymes where the basic oils work on catalyzing the production of antibodies as a result of catalyzing the immune system by the active materials existed in the vegetable oils and then increasing reproduction and differentiation of the immune cells, as well as, increasing the excretion of immune bodies (Tokura *et al*., 1999). Phytochemicals have the ability to increase immunity in the body (*Desai et al.*, 2009). As Youssef (2015) explained that one of the most important functional foods that improve the functioning of the immune system are olive oil and fish oils, Immune proteins also play a vital role in the causes of cancer and chronic inflammation, as it has a pivotal role in the prevention and diagnosis of cancer diseases (Mohmoud *et al*., 2011).

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In accordance to the treatment and medical importance for these vegetable oils and nonexistence of enough local studies about this topic. This study aims at:
Studying the effect of using different concentrations of vegetable oils and comparing them with local commercial oils and showing their effect on the biochemical and enzymatic characteristics of adult male rats.

MATERIALS AND METHODS

Plant oils

The vegetable oils (Grape seed oil, Pumpkin seed oil, Black seed oil, Olive oil) were obtained from Al-Emad Company for Vegetable Oils Production in Mosul/Iraq. As for sunflower oil and corn oil, it was obtained from local markets.

Animals of Experiment

Laboratory animals were obtained from the College of Veterinary Medicine / University of Tikrit. They were adult white male rats, by weights ranging between 170 - 200 g, with a number of (28) animals.
The animals are divided into seven groups, each group containing (4) animals divided as follows:

1- Control group Distilled water was given.
2- The group of animals given grape seed oil at a concentration of 760 mg/kg body weight/day.
3- The group of animals given pumpkin seed oil at a concentration of 250 mg/kg body weight/day.
4- The group of animals given black seed oil at a concentration of 1 ml/kg body weight/day.
5- The group of animals given olive oil at a concentration of 2 ml / kg body weight / day.
6- The group of animals given sunflower oil at a concentration of 500 mg/kg body weight/day.
7- The group of animals given corn oil at a concentration of 10 ml / kg body weight / day.

After the end of the experiment which reached 21 days, the animals are prevented from eating for about 12 hours and then anesthetized by chloroform. Blood is drawn from the heart directly by a medical syringe, about 5- 7ml of blood into two sets of tubes, one of which containing an anticoagulant substance (EDTA) for blood picture analysis and the other free of an anticoagulant, it was centrifuged at 3000 rpm for 15 minutes to obtain serum and stored at -20°C until the analysis is performed (Tietz, 1995).

Blood tests

From the blood collection tubes containing the anticoagulant substance EDTA, the total number of red blood cells (RBC), the total number of white blood cells (WBC), hemoglobin (Hb), granulocyte neutrophil (Gran), lymphocytes (LYM) and platelet (PLT) are all measured using a complete blood Count (CBC) device of the type Celtac (Tietz, 2005).

Estimating the activity of liver enzymes in the blood serum

The activity of the enzyme Aspartat Aminotransferase (AST) and (ALT) is estimated using a British Randox type analysis kit as stated in (Tietz, 2005).

Quantitative Determination of Immunoglobulins (IgA, IgM, IgE,IgG)

Immunoglobulins are estimated using ELISA (Enzyme Linkage Immunue System Assay) as mentioned in Tietz (2005). The kit and solutions are left at laboratory temperature for several minutes to ensure the evaporation of the water on their surface. The determination is carried out by filling the holes in the device plate with an amount of 5 microliters of each of the samples as well as the control sample and left for 15 minutes to complete the adsorption process before being closed. It is then transferred to the device incubator at a temperature of 35 ºC for 72 hours in the case of determination of IgA and IgE globulins and for 96 hours in the case of IgM and IgG type.

RESULTS AND DISCUSSION

The Effect of oral administration of vegetable oil-treated rats in blood images

Table No. (1) shows the effect of oral administration of vegetable oil-treated rats in blood images. The results show a significant increase in the values of RBS, HGB, GRAN, LYM and PLT, which amount to (7.88, 14.30, 2.81, 9.05, 992.50) respectively when adding Pumpkin oil, and a

significant decrease in the above values when adding sunflower oil, which amount to (5.30, 9.85, 1.28, 3.55, 524), respectively. The results also show a decrease in the total number of WBC white blood cells when animals are dosed with essential oils, and the lowest number is when the addition of pumpkin oil is at 9.45 compared with the control groups. It is also noted that numbers increased when corn and sunflower oil are added, compared to the control group.

Table (1): The Effect of oral administration of vegetable oil-treated rats in blood images

Groups	RBC 10^6/ul	WBC 10^3/ul	HGB g/dl	GRAN 10^3/ul	LYM 10^3/ul	PLT 10^3/ul
Control	7.33^{C}	12.05^{B}	12.90^{D}	2.27^{C}	6.40^{C}	845.50^{CD}
Grape seed oil	7.49^{C}	9.65^{E}	13.75^{B}	2.71^{AB}	7.70^{B}	944.50^{B}
pumpkin oil	7.88^{A}	9.45^{E}	14.30^{A}	2.81^{A}	9.05^{A}	992.50^{A}
Black seed oil	6.95^{D}	10.55^{C}	13.05^{C}	2.41^{BC}	7.25^{BC}	909^{C}
Olive oil	7.69^{B}	10.20^{D}	14.55^{B}	2.57^{B}	7.65^{B}	951^{B}
Sunflower oil	5.30^{E}	12.30^{AB}	9.85^{E}	1.28^{E}	3.55^{E}	524^{E}
Corn oil	6.75^{D}	12.40^{A}	11.55^{DE}	1.29^{D}	4.40^{D}	624^{D}

A-E: Different letters in the same column indicate significant differences at the 0.05 probability level

The results indicate that the increase shown in blood images of rats to which pumpkin oil is added is due to the high content of these oils in Omega-3, which improves the health status of rats and increases their immunity through activating the process of phagocytosis and increasing the secretion of cytokines (Calder *et al*., 2011). The high concentration of hemoglobin in the blood and the volume of blood cells packed in the blood serum is also attributed to the content of these oils from vitamins such as vitamin A, thiamine, riboflavin, nicotinamide, vitamin C and minerals such as iron, calcium, zinc, magnesium and amino acids rich in alanine, aspartic acid, glycine and others, which are factors that stimulate the production of blood cells from the bone marrow (Fasuyi, 2006). This is in agreement with what is mentioned by Al-Handal (2020) in that adding pumpkin seed oil to animal diets leads to an increase in the efficiency of food utilization. Pumpkin oil contains Omega 3 which is considered a growth stimulant, and it activates bile increasing the digestion of fats and also increasing the efficiency of digestion and absorption in the intestines leading to better utilization of food and consequently higher levels of hemoglobin, platelets and red blood cells. Results also showed the rise of white blood cells in the groups that corn oil and sunflower oil had been added on, and that because of the rise in numbers of white blood cells that have their defense job against the strange bodies which attack the body and that for what they have of industrial extra materials and industrial antioxidants work on its removal. In addition, white blood cells are considered as the first defense line when the immune system in the body faces the external effects (Chen *et al.*, 2015).

The Effect of oral administration of vegetable oil-treated rats on immune globulins

The results in Table No. (2) show the effect of oral administration to rats treated with vegetable oils on immune globulins. The highest concentrations of immune globulins IgA, IgM, IgE, and IgG are achieved when adding pumpkin oil amounting to (94.51, 82.22, 7.87, 8.36), respectively. The lowest concentrations of IgA and IgM are achieved when adding sunflower oil amounting to (69.84, 50.42) respectively, and the lowest concentrations of IgE and IgG are achieved when adding corn oil with the amounts (3.30, 3.97), respectively.

Table (2): The effect of oral administration of vegetable oil-treated rats on immune globulins

Groups	IgA	IgM	IgE	IgG
Control	82.23^{D}	67.21^{E}	4.32^{D}	5.69^{D}
Grapes seed oil	88.06^{B}	77.82^{B}	6.65^{CB}	7.72^{B}
Pumpkin oil	94.51^{A}	82.22^{A}	7.87^{A}	8.36^{A}
Black seed oil	84.66^{C}	76.80^{C}	5.86^{C}	6.72^{C}
Olive oil	86.36^{CB}	72.48^{D}	6.93^{B}	7.37^{CB}
Sunflower oil	69.84^{F}	50.42^{F}	3.33^{E}	4.11^{E}
Corn oil	72.32^{E}	54.68^{F}	3.30^{E}	3.97^{F}

A-E: Different letters in the same column indicate significant differences at the 0.05 probability level

The results that have been reached indicate the high levels of immunity of rats treated with grape seed oil, pumpkin oil, black seed oil and olive compared sunflower oil and corn oil. This is due to the fact that these oils contain phenolic substances, carotenoids, active substances, unsaturated fatty acids and antioxidants that play a key role in increasing immunity levels (Burstein, 1970).

As for the commercial oils, a decrease in the levels of immunity occurs when rats are given sunflower oil and corn oil. The reason is that these commercial oils are impure and may contain preservatives and artificial antioxidants in high concentrations, which leads to negative effects on the immune globulins. The reason may also be attributed due to the possibility of contamination of these commercial oils with heavy metals, which affects and inhibits the immune response by reducing the production of immunoglobulins such as IgA, IgM, IgG. This makes individuals vulnerable to inflammatory diseases as it increases the susceptibility of the host to bacterial and viral infections and cancers (Metryka *et al.*, 2018).

The effect of oral administration of vegetable oil-treated rats on liver function

The results in Table No. (3) show the effect of oral administration to rats treated with vegetable oils on liver function. The highest concentrations of ALT and AST enzymes are in corn oil and sunflower oil, reaching (30.10, 91.63) IU/liter, respectively, and the least concentration of ALT and AST enzymes is in pumpkin oil reaching (22.99, 83.64) IU/liter, respectively. The reason of oil decreasing when having the basic oils belongs to the ability of these oils on fighting liver injury. Having these oils decreases Liver enzymes because they are anti-inflammatory, antimicrobial and anti-cancer cells, as well as the system is enriched of active materials that improve the immune response and eliminate lever diseases (Zhang *et al*., 2015). As for the occurrence of the rise, it may be attributed to the heart failure & the rising of AST, and that is an evidence for myocardial infarction and angina pectoris because the injured have an elevated enzyme (Crawford *et al*., 2004).

Table (3): The effect of oral administration of vegetable oil-treated rats on liver function analyzes

Groups	ALT IU/L	AST IU/L
Control	28.01^{B}	88.08^{C}
Grapes seed oil	23.77^{D}	84.54^{E}
Pumpkin oil	22.99^{E}	83.64^{E}
Black seed oil	24.63^{C}	85.45^{D}
Olive oil	23.98^{CD}	85.02^{ED}
Sunflower oil	30.06^{BA}	91.63^{A}
Corn oil	30.10^{A}	90.24^{B}

A-E: Different letters in the same column indicate significant differences at the 0.05 probability level

The results indicate a significant decrease in the level of enzymes in the group of rats that are given pumpkin oil. The reason is due to the ability of pumpkin to combat liver injuries . Feeding rats for 21 days with pumpkin oil has reduced the concentration of AST and ALT because it is anti-inflammatory, anti-microbial and anti-cancer cells. Moreover, the oil is rich in carotenoids as it enhances the immune response and reduces liver disease (Zhang *et al.*, 2015). The results agree with Al-Hamdani (2020), as he has shown that giving rats pumpkin oil for 30 days leads to a decrease in the levels of ALT and AST enzymes. As for the significant increase in the levels of ALT and AST enzymes when adding corn oil and sunflower oil, may be due to the possibility that these oils contain high concentrations of saturated fatty acids, which has led to damage to liver tissue due to health problems caused by fat such as high blood pressure and impeding its flow smoothly within the various organs.

Wang *et al.* (2012) indicate that feeding rats with oils containing a high amount of calories leads to an increase in the proportion of saturated fatty acids in the liver, which may reverse liver injury by raising the activity of liver enzymes.

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تأثير الزيوت النباتية على بعض الصفات الفسلجية لذكور الجرذان البالغة

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جامعة تكريت - كلية الزراعة - قسم علوم الأغذية - العراق

الكلمات المفتاحية:
زيت بذور العنب ، زيت اليقطين ، زيت الحبة السوداء ، زيت زهرة الشمس, زيت الذرة ، ، ذكور الجرذان البالغة .

الخلاصة

اجريت الدراسة في البيت الحيواني التابع لكلية الطب البيطري / جامعة تكريت لمعرفة تأثير تراكيز لأربعة انواع من الزيوت النباتية المتحصل عليها من شركة العماد لانتاج الزيوت وهي زيت بذور العنب وزيت اليقطين وزيت الحبة السوداء وزيت الزيتون مع نوعين من الزيوت التجارية المتحصل عليها من الاسواق المحلية وهما زيت زهرة الشمس وزيت الذرة على بعض الصفات الفسلجية لذكور الجرذان البالغة والبالغ عددها (28) حيوانا والتي قسمت الى (7) مجاميع كل مجموعة تضمنت (4) حيوانات. خضعت الحيوانات الى دورة ضوئية انقسمت إلى (12) ساعة ضوء و(12) ساعة ظلام، وثبتت درجة الحرارة عند (2±25) °م , تركت الحيوانات لمدة أسبوع للتأقلم مع الظروف الجديدة وللتأكد من خلوها من الأمراض وأعطيت الغذاء والماء بشكل مستمر عن طريق التجريع الفموي وبكميات كافية طوال فترة التربية.

بينت النتائج إرتفاع في العدد الكلي لكريات الدم الحمراء والهيموكلوبين والخلايا الحبيبية والخلايا اللمفية والصفائح الدموية عند اضافة زيت بذور العنب وزيت الحبة السوداء وزيت اليقطين وزيت الزيتون. وانخفاض في عدد خلايا الدم البيضاء مقارنة مع إضافة زيت زهرة الشمس وزيت الذرة. وكذلك إرتفاع في قيم الكلوبيولينات المناعية IgA و IgM وIgE وIgG عند اضافة زيت بذور العنب وزيت الحبة السوداء وزيت اليقطين وزيت الزيتون، وإنخفاض في قيم إنزيمات الكبد ALT وAST مقارنةً مع إضافة زيت زهرة الشمس وزيت الذرة .