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Effect of sesame oil on some blood parameters and antioxidants in ovariectomized female white rats

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***Abstract*---**This study was conducted to find out the physiological effect of sesame seed oil (Sesamum indicum) in the case of oral treatment on post-menopause and the occurrence of surgical menopause as a result of the removal of the ovaries, by studying the changes in some blood parameters and some antioxidants in the serum of adult female rat pupae. White (Albino Rats), 15 adult rats about three months old, with weights ranging between 160-180 g, were used. The animals were randomly divided into three groups, five animals per group. The first group represented the negative control, which was normal without removing the ovaries, the second represented the positive control in which the ovaries were completely removed, and the third group had the ovaries completely removed and treated orally with sesame oil at a concentration of 4 ml/kg of body weight per day for a period of 30 days. The results of laboratory tests for the blood samples of the completely removed ovaries group compared to the negative control group showed a significant increase (P<0.05) in the number of red blood cells (RBCs) and an insignificant increase)P (0.05<in the volume of pressurized blood cells (PCV) and hemoglobin (Hb), While there was a significant decrease (P<0.05) in the number of white blood cells (WBCs), As for the antioxidants, a significant decrease (P<0.05) in the serum levels of glutathione (GSH) and a significant increase (P<0.05) in the level of malondialdehyde (MDA) was observed. As for the treatment with sesame oil, the tests showed a non-significant increase) P (0.05<in the levels of all the above-mentioned blood parameters, while a non-significant increase) P

(0.05<in the serum levels of glutathione and a significant decrease (P<0.05) in the blood serum levels of malondialdehyde. We conclude from this, that sesame oil has positive effects in the body by stimulating an increase in the production of blood cells and treating oxidative stress by increasing antioxidants.

***Keywords*---**sesame oil, blood, antioxidants, ovariectomized.

Introduction

The demand among peoples for the use of drugs has increased strongly as a result of the huge increase in the population of the world and their frequent exposure to diseases, so synthetic drugs were used instead of natural ones. Medicinal plants have become a source of drugs and active substances that are included in the preparation of drugs in the form of extracts, active substances or raw materials for use in the treatment of many disease conditions such as cortisone, sex hormones, plasma substitute, and others. Among the blessings of God (Almighty and Glory be to Him) over His creation is the presence of medicinal plants that are a food and medicinal source for various diseases because the herbs or their plant parts contain chemicals of great benefit and importance due to their physiological and physiological effects and their therapeutic activity for humans and animals, and phytochemicals are micronutrients and biologically compatible elements. And it has extraordinary efficacy in treating multiple diseases with minimal or no harmful effect (1) (2). Some scientific research has directed the study of medicinal plants in order to investigate the role of antioxidants in these plants in order to prevent oxidative damage resulting from the reactions of radicals. Free in a number of pathological conditions, thus protecting the vital function of the cell (3).

The sesame plant (*Sesamum indicum*) stands out among those important medicinal plants that have several benefits, as sesame belongs to the family Pedaliaceae and is often cultivated in tropical and subtropical regions in the continents of Asia, Africa and South America, and the word "Sesame" is derived from the Arabic word "sesame". (4) It is also called in the Arab countries by this name “Simsim” (5), and it is also known by other names such as: Al-Shamsham, Sheraj or Al-Sirj, Jaljlan and Al-Saleet (6). Globally, it is also known as Sesame or Simsim in Asia and Africa (7). According to prehistoric studies, the cultivation of sesame was discovered in wild groups in southern Asia, and cultivation originated there around 2000 BC, specifically from the time of the Harappan civilization (8).

Materials and Methods

Various devices, tools and chemicals were used in this study, in addition to the ready-made kits obtained in the form of standard kits for the purpose of conducting the required laboratory tests. Experimental animals, represented by female Albino Rats, were three-month-old adult virgins. Approximately, and with weights ranging between 160-180 g, they were obtained from the animal house of the Faculty of Pharmacy at Karbala University. The animals were housed in a miniature animal house that was prepared in the researcher’s house, and the

place was with specifications and laboratory conditions suitable for conducting the experiment, including ventilation, lighting, heating and cooling ..etc., The animals were placed in special rat cages made of plastic, fitted with a clip-on iron cover, and equipped with special water drinking bottles with a nipple at the end. The floor of the cages was spread with a quantity of sawdust, which is replaced from time to time to maintain the cleanliness of the place and the animals. The pelt and the necessary drinking water were also given freely. The animals were left for two weeks to ensure that they are free of various diseases and to adapt to the conditions of the experiment before being subjected to the study, which is the removal of the ovaries and the treatment with sesame seed oil and the physiological tests that accompanied this study.

Sesame seeds were also used in this study, which are available in the local markets of Karbala governorate. Sesame seed oil was used in the experiment of the current study, and this oil was previously extracted according to the standard AOAC method (9), as the method of preparing seeds for the purpose of extraction included several different and sequential steps to extract the oil, this method was summarized by bringing a quantity of dry sesame seeds (seeds weighing 20 g and sizes ranged between 1-2 mm) and exposing them to direct sunlight in order to reduce the moisture content of the seeds and then grinding them well using an electric grinder, and the ground product is placed through a funnel Covered with a cotton tampon and inserted into the Soxhlet extractor, then 250 ml of hexane was poured into the round bottom beaker and connected to the extractor, The condenser was also connected to the extractor, and the rubber hose connected to the inlet of the condenser was connected to a water tap allowing water to flow in and out of the outlet hole, and a heater device was set at 70°C to provide heat to the bottom of the flask placed on the heating plate, with heating continuing for some time, it was observed The presence of a colored solution (sesame oil extract) in the bottle, then filter the resulting oil containing some sesame seed crumbs using a fine sieve, then this pure, pure oil was placed in a clean glass bottle, and this process was repeated several times for the purpose of obtaining the required amount of oil for later use in the process of dosing experimental animals.

The oil dose given to the experimental animals was 4 ml/kg body weight of the animal, which is the appropriate dose that could have the effective effectiveness to perform the purpose for which the oil of this plant was used (10). The treated groups of experimental animals (white rats) were dosed with sesame oil orally and on a daily basis for one month only (while ensuring that the oil reaches the stomach directly) after calculating the exact dose of oil in relation to the animal's body weight. The 15 experimental animals (female white rats) were randomly divided into three groups with five replicates for each group, according to the following description (Fig. 1):

- The first group: is the negative control group called Sham, during which an operation was performed on the animal’s abdomen (opening the skin and abdominal muscles only) without excision of the ovaries and closing the skin in the same way as for the removal of the ovaries, then giving a break for two weeks after the operation and then conducting tests after one month.

- The second group: The positive control group, in which a complete ovarian excision was performed, then a break was given for two weeks after the operation, then tests were performed after one month.
- The third group: is the group treated with sesame oil by direct dosing method to the stomach after a complete removal of the ovaries, as the rats were dosed with sesame oil for one month after completely removing the ovaries and giving a break for two weeks after the operation.

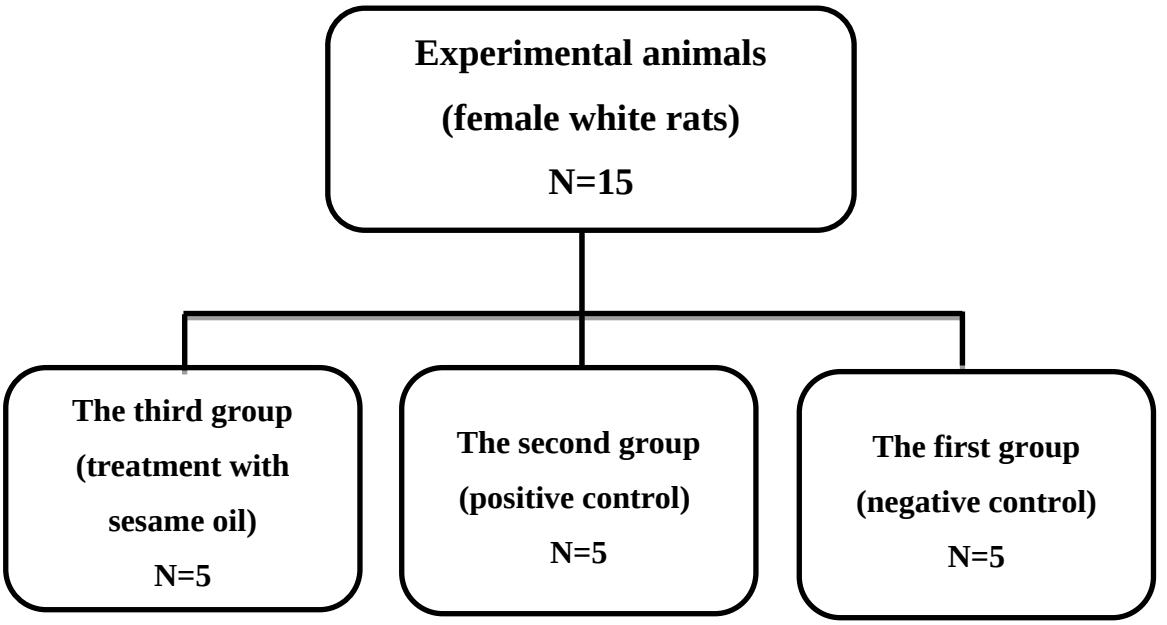

Figure 1. Experimental design

Ovaries were removed from female albino rats under sterile surgical conditions. The animals were anesthetized by general anesthesia by intramuscular injection. The rat was injected with Xylazine at a dose of 10 mg/kg body weight of the animal and ketamine at a dose of 50 mg. kg of body weight of the animal (11). 3-5 minutes after injecting the rat with the anesthetic, the rat was placed in a lying position on the back, then body hair was removed from the abdominal area, the area was cleaned and the area was sterilized with 70% ethyl alcohol. The middle abdomen is below the navel, as the skin, the abdominal muscles and the peritoneum were opened, and the intestines were pushed aside so that the uterus appeared clear. After following the uterus upwards from the right and left sides, the right and left ovaries were easily reached, after which the ovaries were removed using electric cautery, after connecting the uterine tube near the ovary twice with absorbable surgical thread. The abdomen (peritoneum and muscles) was made with absorbable surgical sutures, type Cat Gut 0/3, by continuous suture method. The skin was also sutured with non-absorbable surgical wires type 3/0 and by intermittent sutures (12), and finally the external wound was sterilized with iodine solution to prevent any infection. Ovaries were removed from all experimental groups except for the negative control group (Sham), where only the skin and abdominal muscles were opened (without removing the ovaries), and the area was closed in the same way as before.

After the procedure of removing the ovaries and dosing with sesame oil on the experimental animals, blood was drawn from the heart of the animal directly (after starving it overnight) while it was lying on its back using clean, sterile 5 ml medical syringes. 3 ml of the drawn blood was placed in marked plastic test tubes. Free of anticoagulant and containing gel tubes for the purpose of obtaining a sufficient and pure amount of serum in order to reach the best results, as the serum was separated from the blood mediated by a centrifuge at 3000 rpm for 15 minutes With the use of ordinary plastic tubes (Plain Tubes), in which a certain amount of water is placed for the purpose of calibrating the centrifuge. The clear and free serum from the rest of the blood components was withdrawn by means of a micropipette, then the separated serum was placed in small, dry, marked Eppendorf Tubes. Conducting tests to measure serum levels of the antioxidants glutathione (13) and malondialdehyde (14).

As for the measurement of some hematological parameters represented by counting the number of red blood cells, the number of white blood cells (15), the volume of compressed blood cells and hemoglobin (16), 2 ml of the drawn blood were put into special test tubes of 3 ml capacity containing an anticoagulant substance EDTA Tubes), then the required tests were conducted directly on them in order to obtain the best results, as these blood samples are subject to hemolysis and damage quickly when left for a period of time. The results of the current study experiment were analyzed according to the factorial experiment model and in a completely randomized design (Factorial Experiments with Completely Randomized Design), as the Least Significant Difference (L.S.D.) test was used under the probability level of 0.05, to show the significance of the differences between the different treatments (17).

Results and Discussion

1. Measurement of the red blood cell count (RBC):
   The results showed a significant increase ($P<0.05$) in the number of red blood cells in the case of complete removal of the ovaries compared to the negative control group (Sham), while the results showed a non-significant increase ($P>0.05$) in the number of red blood cells in the case of group treated with sesame oil compared to the group of the positive control.
2. Measurement of White Blood Cell Count (WBC):
   The results showed that there was a significant decrease ($P<0.05$) in the number of white blood cells in the case of complete removal of the ovaries compared to the negative control group (Sham), while the results showed a non-significant increase ($P>0.05$) in the number of white blood cells in the case of group treated with sesame oil compared to the group of the positive control.
3. Measurement of pressurized blood cell volume (PCV):
   The results showed a non-significant increase ($P>0.05$) of PCV in the positive control case (represented by complete removal of the ovaries) compared to the negative control group (Sham). The results also showed an insignificant increase ($P>0.05$) in the volume of pressurized blood cells in the case of group treated with sesame oil compared to the group of the positive control.
4. Hemoglobin (Hb) Level Measurement:

The results showed a non-significant increase (P>0.05) in hemoglobin level in the positive control case (represented by complete removal of the ovaries) compared to the negative control group (Sham). The results also showed an insignificant increase (P>0.05) in the hemoglobin level in the case of group treated with sesame oil compared to the positive control group.

5. Glutathione (GSH) level measurement:
   The results showed a significant decrease (P<0.05) in the level of glutathione in the positive control case (represented by complete removal of the ovaries) compared to the negative control group (Sham). The results also showed that there was a non-significant increase (P>0.05) in the level of glutathione in the case of group treated with sesame oil compared to the group of the positive control.
6. Malondialdehyde (MDA) level measurement:
   The results showed a significant increase (P<0.05) in the level of malondialdehyde in the positive control case (represented by complete removal of ovaries) compared to the negative control group (Sham). The results also showed a significant decrease (P<0.05) in the level of malondialdehyde in the group treated with sesame oil compared to the positive control group.

Table 1
Effect of ovariectomy and treatment with sesame oil on some blood parameters and antioxidants in female white rats

Parameter / (Unit)	Sham	Ovariectomized females	Ovariectomized females treated with sesame oil
RBC / (10^12/L)	A 0.08 ±8.17	0.09 B ±8.55	0.08 B ±8.65
WBC / (10^9/L)	0.28 A ±4.7	0.17 B ±3.4	0.25 B ±3.7
PCV / (%)	0.96 A ±47.9	0.90 A ±49.3	1.25 A ±49.8
Hb / (g/dL)	0.29 A ±14.5	0.27 A ±14.9	0.38 A ±15.1
GSH / (µmol/mL)	3.32 A ±66.3	2.17 B ±30.8	1.87 B ±34.9
MDA / (µmol/mL)	0.90 A ±17.6	1.66 B ±55.7	1.60 C ±52.0

- The values represent the mean of five animals ± Standard Error.
- Different capital letters in the horizontal direction represent the presence of significant differences at the level of probability (P<0.05).

The removal of one or both ovaries from the experimental animals (female albino rat) led to an increase in the number of red blood cells (RBC), and thus an increase in the amount of hemoglobin (Hb) in the blood, as well as an increase in the volume of pressurized blood cells (PCV), while this was offset by a decrease in white blood cell (WBC) count. The results of the current study were completely in agreement with the results of the study carried out by Bracamonte *et al.* (18), which indicated (through their study of a number of blood parameters of female pigs after ovaries removal) that an increase in the number of red blood cells, an increase in the amount of hemoglobin in the blood and an increase in the size of cells Compression and a decrease in the number of white blood cells (lymphocytes). The process of removing the ovaries (according to the researcher's opinion) has contributed to an increase in growth factors in the blood plasma,

and this is what caused an increase in the number of RBCs, which led to an increase in blood viscosity in response to the wound caused by removing the ovaries. As for the decrease in the number of WBCs in the blood, it was It is attributed to the migration of these cells from the bloodstream through the walls of blood vessels to the site of the wound (the site of oophorectomy) to treat cases of infection that may occur as a result of the removal process (18) (19).

The changes in the studied hematological parameters of ovariectomized experimental animals after sesame oil dosing (represented by an increase in the number of red blood cells, hemoglobin, the volume of compacted cells and the number of white blood cells), may be attributed to stimulating the bone marrow to increase the formation of blood cells (blood cells) due to the containment of the oil. Sesame contains vitamin B12, and the presence of some important elements such as iron (Fe), copper (Cu) and cobalt (Co) in the composition of sesame oil, leads to an increase in the concentration of hemoglobin in the blood (20). On the other hand, sesame oil increases the effectiveness of white blood cells and lymphocytes and thus activates immune cells due to the role of this antioxidant oil, as a result of containing antioxidants such as phenols, vitamins C and E (which work to break down free radicals), which leads to the protection of the membranes of Cells from Crash (21).

GSH is one of the most important antioxidant defense systems, playing a large and important role in protecting cells, tissues and organs from oxidative stress. The decrease in the level of estrogen in the serum of ovarian groups (as we have noticed) increases the generation of oxygen free radicals and active types of oxygen, and thus leads to lipid peroxidation, which results in the formation of endocyclic peroxides, and it also works to break down the acyl chain in fats. The phospholipids present in the cell membrane, leading to changes in the membrane dynamics and its destruction, thus reducing the antioxidants, including GSH, and thus accompanied by a rise in the level of malondialdehyde (MDA) in the blood serum, which is an indicator of oxidants that destroy cell membranes in the body (22) (23) (24). The results of the current study showed that the process of removing the ovaries of experimental animals (female albino rat) led to a significant decrease in the level of GSH in the blood serum. It also agrees with the study of Al-Saadi (26) on rabbits, which indicated a decrease in the level of GSH and an increase in the level of MDA in the serum of ovariectomized rabbits, and the accompanying decrease in the level of estrogen. These changes show the importance of estrogen in the process of regulating and stabilizing the level of glutathione in cells and tissues and inhibiting the oxidation of lipoproteins and lipid peroxidation in animal tissues (27).

The results of the current study also agree with the study done by Kaim (28) on rabbits, which indicated a decrease in the level of GSH (which is a non-enzymatic antioxidant) in the blood serum of ovaries removed, and the deficiency in ovarian hormones (estrogen and progesterone) has a great relationship with the failure to balance the state of oxidative stress, in other words, the imbalance between oxidants and antioxidants, and this was also accompanied by an increase in the level of MDA in the blood serum of those ovaries removed. The significant decrease in the level of glutathione in the blood serum of female rats exposed to oxidative stress can be attributed to the oxidation of glutathione or a decrease in

its synthesis, as glutathione has an important role in inhibiting the types of active oxygen molecules that cause cell destruction or catabolism. The important biological processes, including the building of protein and nucleotides, and it also contributes to the effectiveness of some enzymes through its currency as a substrate) or as an enzymatic companion for some enzymatic processes in the cell (29), so a decrease in its level in the serum is an indication of an increase in oxidative stress.

Also, the significant increase in the level of malondialdehyde in the serum of ovarian female rats affects the ability of the system to get rid of oxidants and remove them, and thus to reduce the levels of GSH, and this result is consistent with the study of Al-Saadi (26) and Kaim (28) on rabbits, which indicates that The sequence that occurs in the case of antioxidants for different tissues as a result of an increase in lipid peroxidation, as in the case of removal of the ovaries, and thus a decrease in estrogen, which is considered an antioxidant. The increase in the level of glutathione and the accompanying decrease in the level of malondialdehyde in the blood serum of female rats subjected to oxidative stress by ovary removal after dosing with sesame oil, may be due to the fact that this oil represents an important oxidizing factor, which can be attributed to the presence of anti-oxidants. endogenous oxidation {sesamol, sesamolin, sesamin} and tocopherols, and sesame oil has superior oxidative stability despite its high level of unsaturation; This quality may be due to the presence of gamma-tocopherol, lignans, and antioxidants (30) (31).

This oxidative role of sesame oil was indicated by Woo *et al.* (32) in a rat study, in which the antioxidant effects of bioactive compounds enriched with sesame oil (such as lignans and tocopherol) were demonstrated, and the important role of sesame oil in reducing or mitigating Kidney damage caused by a high-fat diet by suppressing oxidative stress and inflammation. The role of sesame oil as an antioxidant may be attributed (according to the researcher) to the fact that this oil contains antioxidants such as phenols, vitamins C and E (which work to break down free radicals), and this leads to the protection of cell membranes from breaking down.

Conclusion

The results of our study experiment indicate that sesame oil has positive effects in the body by stimulating increased production of blood cells and treating oxidative stress conditions by increasing antioxidants.

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