



# An Investigation of the Biological Effects of the Consumption of Various Edible Oils on Reproductive and Hepatic Parameters in Pregnant Rats

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## ABSTRACT

**Background:** The purpose of this study was to evaluate the effects of different edible oils on reproductive performances and liver functions in female albino wistar rats with hyperlipidemia by analyzing factors including pregnancy weight gain, estrous cycle length, fertility rate, pups number, offspring weight, liver weight, enzyme activities and oxidative stress.

**Methods:** The 56 rats were divided into seven different experimental groups (Control, olive, coconut, palm, soybean, sunflower and flaxseed), respectively.

**Result:** All groups displayed comparable pregnancy weight gain, ranging from 11.25±0.49 g to 18.02±0.52 g. Meanwhile, the uterine weights of the soybean and palm oil groups were lower than those of the control, at 1.88±0.23 g and 1.87±0.26 g, respectively. While the palm and soybean groups had the lowest fertility rates of 64.11±4.41 % and 67.85 ±4.91%; the coconut, flaxseed and olive oil groups had the highest offspring weights of 6.77±0.47 g, 6.56±0.47 g and 6.43±0.47 g, respectively. Rats that received flaxseed oil produced the highest number of pups, 9.34±0.43 and the least amount of liver weight loss 5.44±0.44 g, suggesting possible advantages for the liver and reproductive system. Edible oils had varying effects on the activity of liver enzymes in obese rats. Oxidative damage was encouraged by coconut and palm oils. Olive and flaxseed oil groups had the highest increase of superoxide dismutases (SOD) and catalase (CAT) activities with the lowest malondealdehyde (MDA) levels. In hyperlipidemic female rats, flaxseed and olive oils generally enhanced the reproductive efficiency and liver function suggesting great protection against metabolic stress.

**Key words:** Antioxidant enzymes, Edible oils, Hyperlipidemia, Liver function, Oxidative stress, Rats, Reproductive performance.

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## INTRODUCTION

Being the second-largest source of energy after carbohydrates, lipids are a crucial part of the diet. Clinical findings have demonstrated a strong correlation between female infertility and obesity as the detrimental effects on cardiovascular disease, ovaries, obesity impairs reproductive processes and raises systemic inflammation by Al-Eisa *et al.* (2024). Numerous strategies were employed to address obesity, such as exercise, calorie-restricted lifestyle, bariatric surgery and pharmacological medicines by Basheer *et al.* (2023). Edible oils contain vital fatty acids and have both direct and indirect anti-inflammatory properties by Al-Qahtani *et al.* (2025). Numerous oils are used in gynecology to hasten the cervix's ripening, which shortens the labor period, lowers the risk of postponed pregnancies and relieves premenstrual syndrome and dysmenorrhea by Reddy *et al.* (2022). Furthermore, protective roles have been demonstrated across the processes of differentiation, reproduction regulation and proliferation by Chen and Schmidt (2026).

There is plenty of evidence that malnutrition has a major impact on fat metabolism and is a contributing factor to weight gain by Morsy *et al.* (2022). Therefore, significant deficits and changes in the metabolism of essential fatty acids (EFAs) are linked to high-fat and low-protein malnutrition in rat development by Baazeem *et al.* (2024). Olive oil that is high in monounsaturated fatty acids (MUFAs) offers anti-atherosclerosis qualities. Linoleic acid, as one of the necessary polyunsaturated fatty acids (PUFAs), is abundant in these oils. Due to their high PUFAs content, sunflower and corn oils are regarded as risk factors for the development of free radicals. Dietary fats have a big impact on inflammation and how it progresses. EFAs are necessary for the offspring's healthy growth in rats by Morsy *et al.* (2022). Inadequate protein and energy intake during pregnancy resulted in a loss of heart muscle mass proportional to the total body mass lost and altered cardiac function. In an intrauterine adhesion rat model, evening primrose oil improved pregnancy outcomes, reduced inflammation and fibrosis by Gutiérrez *et al.* (1994) and Gomez-Velez *et al.* (2024). In rats modeled for polycystic ovarian disease, clove oil possesses certain biochemical and histological characteristics as well as autophagy markers. In pregnant rats with diabetes, walnut oil has positive effects on preventing hyperlipidemia and pro-oxidant states by Yadav *et al.* (2024). Different dietary oils have different biological impacts on hepatic and reproductive processes during pregnancy. Olive oil promotes liver function and improves reproductive results since it is high in monounsaturated fats and antioxidants. Although its saturated fat concentration may alter hormone balance, coconut oil's high medium-chain triglyceride content affects hepatic metabolism. Although tocotrienols found in palm oil have antioxidant qualities, consuming too much of them can cause inflammation in the liver. Both sunflower and soybean oils are important for hormone

synthesis but can upset the hepatic lipid balance if omega-3 intake isn't balanced. On the other hand, flaxseed oil, is linked to decreased hepatic oxidative stress and enhanced reproductive performance by Sun *et al.* (2020); Soltani *et al.* (2023); Neykhonji *et al.* (2024).

Finding the most beneficial edible oil with the best qualities is crucial in this respect to identify the best edible oil for improving newborn development metrics and maternal reproductive performance when high-fat-induced metabolic stress is present. Evaluating pregnancy weight gain, estrous cycle length, fertility rate, pups number, offspring weight, liver weight, enzyme activities and oxidative stress were among the factors used in this study to assess the effects of various edible oils on reproductive performances and liver functions in female Albino Wistar rats with hyperlipidemia.

## MATERIALS AND METHODS

### Experimental design and edible oils

Female rats were used in an efficacy study called the "treatment trial" to examine the potential benefits of edible oils for certain reproductive and hepatic functions. Adult male rats weighing between 300 and 340 grams (N=7) and mature female Albino Wistar rats weighing between 230 and 240 grams and aged between 67 and 77 days (N=56) were selected from the Animal Holding Unit of the Biological Science in Jeddah, Saudi Arabia. Females were kept in standard conditions in plastic cages that were one square foot, had a temperature range of 27-30°C, a relative humidity of 60 % and had a normal 12-hour darkness and night cycle. One group of eight virgin females with a single male was properly kept in a single cage and labeled to prevent uncertainty regarding the pups in the event of an early delivery. It was allowed for male and female rats to mate 1-3 days. Daily vaginal smears performed at 9:00 A.M. to check for the presence of sperm by applying water-moistened cotton (day 0 = day of copulation) were used to confirm copulation. Gestational day 0 was the day that the presence of sperm in the smear was regarded as proof of copulation.

Urine, feces and food debris were removed daily to prevent contaminating the food and water. Rats were given basic chow (20-23% protein, 4-6% fat, 5-8% crude fiber and the remainder of complex carbohydrates such as corn, wheat and oats) from Petland, Jeddah. The rats were fed the basic chow diet for two weeks to acclimate which contained all of the food ingredients and had complete access to tap water during the trial. In order to cause hyperlipidemia in female rats, they were then given a high-fat diet for 21 days. Rats given a high-fat diet often displayed a number of physical signs that indicate the beginning of hyperlipidemia. Weight gain is typically the result of increased fat deposition. Additionally, a larger, paler and heavier liver may be a sign of hepatomegaly or fatty liver changes. Affected rats may show signs of metabolic stress, such as lethargy or decreased activity, in their behavior.

Sometimes a single may also be noticed as changes in the quality of the fur, such as greasiness, dullness, or an untidy appearance by Rabail *et al.* (2024). Several types of edible oils have been used in the current experimental work. The Saudi Arabian olive oil (Al Jouf), coconut oil were purchased from India's Parachute, flaxseed oil from Saudi Arabia's LuLu, palm, soybean and sunflower oils from Saudi Arabia's (Afia) from a local market in Taif City, Saudi Arabia. The commercial brands that were utilized were widely accessible food-grade goods that were meant for human consumption. The oils were not purchased from a particular manufacturer because they were sourced locally from retail markets; rather, they are representative of the normal consumer-grade oils that were accessible in the area at the time of the study.

The rats were divided into seven different experimental groups at random (control, olive, coconut, palm, soybean, sunflower and flaxseed), respectively. In order to ensure that all diets were isocaloric and isonitrogenous, experimental diets were made by adding 7% of each oil (olive, coconut, palm, soybean, sunflower, or flaxseed) to a semi-purified basal diet based on the AIN-93 formulation. The ingredients were carefully combined, pelleted and kept at 4°C in airtight containers to prevent lipid oxidation. Fresh batches were made every week for the duration of the 40-day feeding period.

#### Gavage feeding

Control group only received an oral dose of distilled water (10 mL/kg) instead of oil consumption. From the first day of pregnancy to the end of the 21-day gestation period, edible oil supplementation was started. Neither before mating nor throughout lactation was there any oil treatment. The dam's esophagus received the various edible oils directly through a blunted, 3-in, 18-20 gauge at a rate of around 0.4 mL/100 g B.W. once a day by a curved stainless steel needle specifically created for this use (Perfectum, 7916, CVD) by Lashin *et al.* (2020). As is customary, each dam was observed for 15 minutes following gavage feeding for symptoms of discomfort or hard breathing. The summary of the study is presented in Fig 1.

#### Pregnancy weight gain

In rats, physiological changes such as increased uterine mass, fetal and placental development and dam adipose tissue accumulation are reflected in gestational weight gain. The weight gain in the pregnancy period was measured by weighing the female rats before mating (pre-pregnancy weight) and comparing it to their weight at a particular point during gestation, usually close to term (e.g., gestational day 20 in at the 21<sup>st</sup>-23<sup>rd</sup> day of pregnancy) to determine pregnancy weight gain in rats. Grams (g) are typically used to express the outcome. To minimize variability, it's critical to maintain consistent weighing conditions (e.g., same time of day, empty stomach). In reproductive research, this measurement aids in evaluating the health of the female rats, the growth of the

fetus and the results of experimental therapies by Daidj and Lamri-Senhadj (2021).

#### Estrous cycle length

Daily vaginal cytology was applied to track the cycle's stages over a minimum of two weeks to determine the length of the estrous cycle in rats. Proestrus, estrus, metestrus and diestrus are the main four phases that process the rat estrous cycle and they typically last four to five days. Every day, vaginal smears were collected and examined under a microscope to identify the distinct cell types present. This made it possible to determine each stage. Record the time between two consecutive occurrences of the same stage, usually estrus, when the female was sexually receptive, to determine the length of the cycle. An accurate average estrous cycle length for that particular rat can be obtained by repeating this across several cycles by Chaitra *et al.* (2020).

#### Fertility rate

The number of female rats that became pregnant after mating was calculated and divided by the total number of mated female rats to estimate the fertility rate. This value was then multiplied by 100 to represent the outcome as a percentage by Ramaiyan *et al.* (2021).

#### Pup number and offspring weight

The total number of pups born in a single litter was used to determine the size of the litter in rats. If relevant, this covered both live and stillborn pups. The computation entails checking on the expectant mother until parturition, or delivery and counting the pups after delivery. Pups number equaled the total number of pups born divided by the number of litters by Hilakivi-Clarke *et al.* (1997). Rat offspring weight was determined by weighing each pup separately using a precision digital balance after birth.

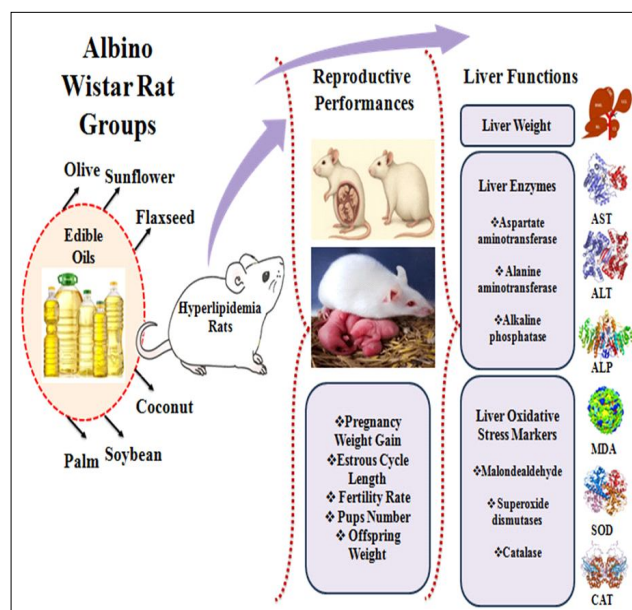


Fig 1: The summary of the experimental work.

### Euthanasia and organ harvesting

Since the rat's gestational cycle is generally 21 days, we delivered the fetuses by gestational delivery on the twenty-first day to avoid spontaneous delivery and the pups' nursing. In order to get samples of reproductive and hepatic tissue, all pregnant females were euthanized on gestational day 21, just before parturition. The dams were put to anesthetization by thiopental sodium (40 mg/kg) and they were then guaranteed to die by creating a pneumothorax by slicing through the chest wall with a tiny pair of scissors by Yadav *et al.* (2024). After using scissors to access the chest cavity and gather the livers and uteruses, the heart was severed to ensure death. A Shimadzu AX 200 analytical scale was used to determine the organs' weight, which was then expressed as (g/100 g). Before being drained using filter paper, the organs were carefully rinsed twice with ice-cold phosphate-buffered saline (PBS pH 7.4). Tissue samples were mechanically homogenized in ice-cold PBS to preserve enzyme activity and avoid protein denaturation. The homogenates were centrifuged for 15 minutes at 4°C and 10,000 rpm and the supernatants were collected for further biochemical investigations, including evaluations of hepatic functions. The homogenates were stored at -20°C to preserve their integrity until they were used again.

### Oxidative stress

For the biochemical analysis, liver tissues were kept at -20°C. Malondealdehyde (MDA), superoxide dismutases (SOD) and catalase (CAT) activities were measured in each group in order to assess oxidative stress. Following 50 mg of tissue homogenization in 0.5 mL of cold lysis buffer (pH 7.4), the supernatant was extracted by centrifugation and utilized for assay and estimation by Wojciechowski *et al.* (2010). The measurements were carried out according to the manufacturer's instructions using ELISA kits (Sigma, MO, USA).

### Liver enzymes

In both clinical and laboratory experiments, liver enzymes are frequently employed as markers of hepatic function and liver injury since they are essential to metabolic processes. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured colorimetrically according to the manufacturer's instructions using ELISA kits (Sigma, MO, USA) and expressed as (U/L) by Baazeem *et al.* (2024).

### Statistical analysis

All of the results were expressed using the mean and its standard errors. The one-way ANOVA was used to compare the means of all experimental groups for each continuous variable that was measured, including organ weights, inflammatory markers and biochemical markers. Each treatment group was carefully compared to the control group for these characteristics using Dunnett's t-test.

Following a significant ANOVA result, statistically significant effects were further investigated. At  $p < 0.05$ , statistical significance was established. For the statistical studies, GraphPad software version 3.0 was utilized.

## RESULTS AND DISCUSSION

### Pregnancy weight gain

This study emphasizes how crucial a dam's nutrition is to both her health and her offspring's development. Rat models are useful for causing obesity in experiments since they gain weight rapidly when fed high-fat meals. During the study, neither experimental group suffered any adverse effects or lost any members. The same amount of food was still consumed by each treatment group. Every week, the impact of feeding different edible oils on body weight gain increased within each group.

Both control and other dams consumed various edible oils and detected comparable amounts of weight during late pregnancy, from 11.25 g to 18.02 g, even though hyperlipidemic dams were noticeably heavier at conception and during the pregnancy. Pregnancy weight gain is shown in Fig 2. The coconut group reported the maximum weight gain of 18.02 g, followed by flaxseed, olive and palm groups, 17.56 g, 17.27 g and 16.78 g, respectively. On the other hand, the soybean and sunflower groups presented a reduction in weight gain compared with the control group of 15.22 g, 15.41 g and 11.25 g, respectively.

Pregnancy weight gain is intrinsically intended to increase, partly because of fetal bulk, maternal fat deposition and changes in the mother's physiology (such as blood volume expansion) by Baazeem *et al.* (2024). These results were in line with Salem (2015), who studied the effects of olive oil feeding on the pregnancy period. It has been demonstrated that the bioactive and antioxidant substances found in oil prevented rats from gaining extra

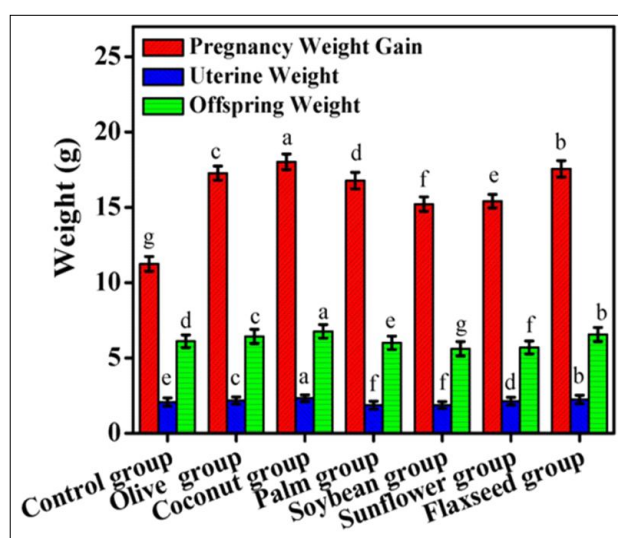


Fig 2: Effect of edible oils on pregnancy weight gain, uterine weight and offspring weight.

weight by Alim *et al.* (2024). Therefore, active intervention techniques may be needed to restrict maternal body weight.

#### Uterine weight

Edible oil consumption after lactation may have a major physiological impact on the uterus and other reproductive organs. The coconut group reported the maximum uterine weight of 2.33 g, followed by the flaxseed, olive and sunflower groups, 2.25 g, 2.18 g and 2.14 g, respectively. On the other hand, the soybean and palm groups presented a reduction in uterine weight compared with the control group of 1.88 g, 1.87 g and 2.08 g, respectively, Fig 2.

According to earlier research, fluid imbibitions may also contribute to the rise in uterine weight by O'Connor *et al.* (1996). Variations in uterine weight have been associated with dietary intake of specific edible oils, such as palm, soybean, or sunflower oil, in postnatal research using rat models. It is believed that these alterations were caused by the oils' fatty acid content, which can alter inflammatory reactions and estrogenic activity by Shang *et al.* (2017). Saturated fats (SFs) can cause hormonal abnormalities and changes in lipid metabolism, while diets high in omega-6 polyunsaturated fatty acids (n-3 PUFAs) may encourage the growth of uterine tissue because of their function in prostaglandin synthesis. Depending on the kind and quantity of edible oil consumed, high consumption during critical developmental times has been proven to either increase or decrease uterine weight. According to these results, dietary lipids may have an impact on the reproductive health of the offspring *via* endocrine and epigenetic mechanisms by Hausman *et al.* (1991).

#### Offspring weight

The coconut group reported the maximum offspring weight of 6.77 g, followed by the flaxseed and olive groups, 6.56 g and 6.43 g, respectively. On the other hand, the soybean and sunflower groups presented a reduction in offspring weight compared with the control group of 5.61 g, 5.70 g and 6.11 g, respectively, (Fig 2).

In rats, the offspring weights were significantly influenced by the edible oils that the dam consumed throughout pregnancy and nursing. Fetal and neonatal development could be greatly impacted by the kind and composition of dietary fats, especially the ratio of SFs, MUFAs and PUFAs. In contrast, oils high in omega-6 PUFAs, such as those present in corn and sunflower oils, can cause altered growth patterns and lower birth weights in offspring by Hausman *et al.* (1991). Walnut oil enhanced the offspring growth in diabetic pregnant rats induced by streptozotocin administration by Sun *et al.* (2020). It was noted, however, that incorporating olive oil, PUFAs and other n-3 sources could help to increase the weight of alcohol-exposed offspring through both prenatal and postnatal supplementation strategies by Yadav *et al.* (2024). As well as another study demonstrating no effects by Heras-Molina *et al.* (2021). The different sources and concentrations of n-3 sources and PUFAs supplemented, along with potential

lifestyle implications, could account for these incongruences. While in humans, obese females are the most probable to have macrosomic children, in hyperlipidemia, rats have consistently shown that newborns exhibit unchanged body weight by Heras-Molina *et al.* (2021). This effect has not yet been adequately explained. Maternal protein intake could be one possible explanation. Therefore, further investigation is necessary before making any recommendations. These results imply that the quality of oil consumed by the dam had a significant impact on the postnatal growth trajectory of the rat offspring.

#### Estrous cycle length

In female rats, the estrous cycle is a hormonally controlled reproductive process that can be affected by nutritional factors, especially the type and composition of dietary fats. As the main sources of dietary lipids, edible oils can influence endocrine function by impacting the synthesis and signaling pathways of steroid hormones. It has been demonstrated that the types of dietary edible oils affect the reproductive physiology in female rats, resulting in changes to the estrous cycle.

The olive group had the same estrous cycle length as the control group, 4<sup>th</sup>-5<sup>th</sup> day. Coconut and flaxseed groups had the same estrous cycle length as the 3<sup>rd</sup>-4<sup>th</sup> day. In addition, palm and sunflower groups had longer estrous cycle lengths during the 5<sup>th</sup>-6<sup>th</sup> day, while the soybean group detected the longest estrous cycle length in the 6<sup>th</sup>-7<sup>th</sup> day, (Table 1).

A complex interplay of hormones, including estrogen, progesterone, luteinizing and follicle-stimulating hormones by Volk *et al.* (2017). These hormones can be influenced by nutritional fat intake. Research has shown that diets rich in PUFAs, particularly omega-6 oils such as sunflower, soybean and corn oil, can interfere with hormonal regulation and result in irregular or extended estrous cycles by Panos and Finerty (1954). In contrast, the intake of edible oils abundant in n-3 as fish and flaxseed oils has been linked to enhancements in estrous cyclicity and ovarian function. This improvement is possibly attributed to these oils' anti-inflammatory effects and positive influence on hormone balance. Moreover, a diet rich in SFs may damage the hypothalamic-pituitary-gonadal axis, leading to cycle disturbances or anestrus in certain instances. It has also been linked to irregular cycles and prolonged diestrus phases, due to detrimental effects on ovarian morphology

**Table 1:** Effect of edible oils on estrous cycle length.

| Groups        | Estrous cycle length (Days)      |
|---------------|----------------------------------|
| Control       | 4 <sup>th</sup> -5 <sup>th</sup> |
| Olive oil     | 4 <sup>th</sup> -5 <sup>th</sup> |
| Coconut oil   | 3 <sup>rd</sup> -4 <sup>th</sup> |
| Palm oil      | 5 <sup>th</sup> -6 <sup>th</sup> |
| Soybean oil   | 6 <sup>th</sup> -7 <sup>th</sup> |
| Sunflower oil | 5 <sup>th</sup> -6 <sup>th</sup> |
| Flaxseed oil  | 3 <sup>rd</sup> -4 <sup>th</sup> |

and follicular development, in case of palm oil was consumed in large amounts by Maarouf *et al.* (2019). The results indicate that the quality of edible oil consumed can have a major effect on rats' reproductive cycling, fertility and health.

### Fertility rate

Both the control group and those consuming edible oils exhibited comparable success rates for initiating and retaining pregnancy. During the time before conception, hyperlipidemia can have an impact on body weight and fertility indices by Ramaiyan *et al.* (2021).

The hyperlipidemia case exhibited a lower percentage of fertility except with the flaxseed group, which had the highest fertility rate among the hyperlipidemic female rats, at 85.02%, while the coconut group detected a similar fertility rate as the control group, 81.21% and 82.55%, respectively. Olive and sunflower groups had lower fertility rates of 74.05% and 73.89%, respectively. Palm and soybean groups had the lowest fertility rate, at 64.11% and 67.85%, respectively, (Fig 3).

Although the fertility and pregnancy index decreased in all hyperlipidemic groups, n-3 from flaxseed oil effectively countered changes induced by the hyperlipidemia. Furthermore, it was well established that dietary fat had a considerable impact on endocrine responses that influenced fertility indices. Research has shown that soybean and hydrogenated vegetable oils hamper ovarian function in female rats, resulting in extended estrous cycles, diminished fertility and a rise in embryonic mortality. The aforementioned oils were associated with ovarian atrophy and the development of follicular cysts, while trans-fatty acids had a detrimental impact on fertility performance in Wistar rats. These specific effects involved a reduction in litter size, changed sperm morphology and physiological changes indicating probable endocrine-disrupting impacts by Litvinova and Fedorchenko (1994). Conversely, research involving male rats showed that diets enriched with flaxseed and sesame oils, which are high in PUFAs, resulted in elevated levels of testosterone, follicle-stimulating hormones and luteinizing hormones. These oils increased sperm counts and improved semen quality, indicating positive effects on male fertility.

### Pups number

The data for pups were presented as a litter mean, with each litter serving as the experimental unit. The effect of edible oils on the number of pups differs based on the specific type of oil and its fatty acid contents. An excessive intake of fat can negatively affect fertility efficiency and the number of pups by Shaw *et al.* (1997).

The flaxseed group reported the maximum number of pups as 9.34 pups, followed by the coconut and sunflower groups, 9.01 and 8.66 pups, respectively. Olive and soybean groups recorded similar values of 8.10 and 8.11 pups, respectively. On the other hand, the palm group presented a reduction in the number of pups compared

with the control group of 6.33 and 7.37 pups, respectively, (Fig 4).

A similar value of the number of pups was detected after the consumption of olive oil due to the presence of n-3 PUFAs (mainly alpha-linolenic acid, ALA) by Yadav *et al.* (2024). Agiang *et al.* (2015) have shown that rats whose diets were supplemented with sesame oil had a greater number of pups than those in the control groups. The rise in the numbers of pups was linked to higher levels of estradiol and luteinizing hormone, indicating that the phytoestrogens in sesame oil may have improved fertility by Shaw *et al.* (1997). Pups of rats that consumed fish oil during gestation and nursing had lower birth weights and diminished postnatal growth. Several pups were not directly impacted, yet the modified metabolic adaptations in the offspring suggest that high levels of PUFAs may affect developmental

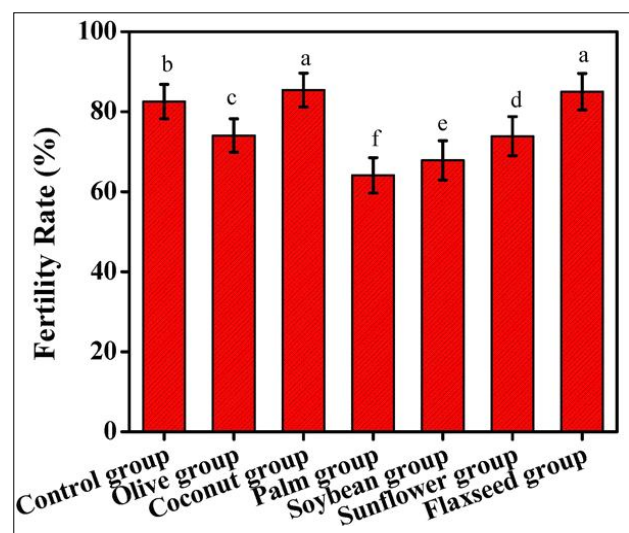


Fig 3: Effect of edible oils on fertility rate.

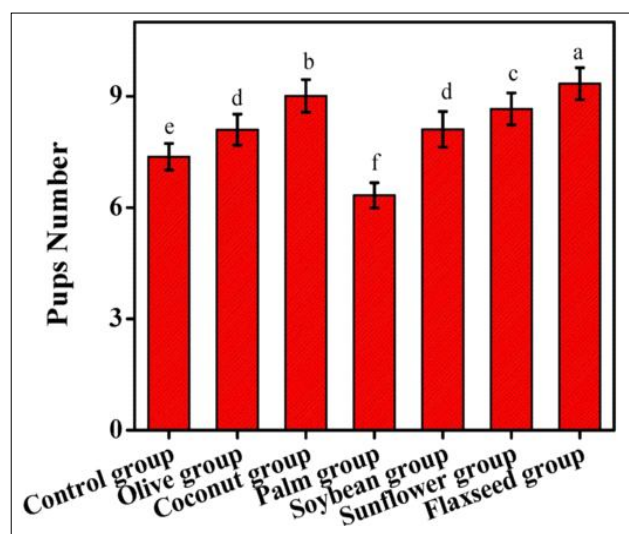


Fig 4: Effect of edible oils on pups number.

outcomes by Jiménez *et al.* (2017). The composition of fatty acids in foods may affect various aspects of the reproductive process, such as oocyte maturation, ovulation timing and chemoattractant production by Albert *et al.* (2016). Prostaglandin synthesis by oocytes could be a potential mechanism behind poor implantation and unfilled embryos by Ramaiyan *et al.* (2021).

### Liver weight

The hyperlipidemic female rats used in this 42-day bio-efficacy investigation had fatty livers. Rats that consume a fatty diet become obese as a result of increased food consumption, body weight and liver lipid storage by Chaitra *et al.* (2020).

The effect of edible oil consumption on rats' livers is displayed in Fig 5. The palm group reported the maximum liver weight of 6.56 g, followed by the coconut and soybean groups, 6.28 g and 6.24 g, respectively. The olive oil group detected a similar value to the sunflower oil group, at 5.99 g and 5.98 g, respectively. On the other hand, the flaxseed group presented the lowest reduction in liver weight of 5.44 g.

Rats fed with olive oil showed a comparatively smaller rise in liver weight than rats fed with coconut oil, indicating that MUFAs may have a preventive effect against hepatic steatosis by Salem *et al.* (2015). Hepatomegaly and increased liver weight linked to fatty infiltration and oxidative stress were observed in rats given soybean oil by Deol *et al.* (2015). When compared to the control group, long-term consumption of canola and flaxseed oils did not significantly change liver weight, indicating a neutral hepatic effect under typical feeding conditions by Atefi *et al.* (2018). In fact, n-3 FAs aid in regulating inflammation and metabolic health and a diet high in PUFAs causes a notable decrease in liver fat accumulation by Chaitra *et al.* (2020).

### Liver enzyme

The effects of various edible oils on the activities of liver enzymes as ALT, AST and ALP, which are indicators of hepatic function and damage, in obese rats have been the subject of numerous studies by Baazeem *et al.* (2024).

The palm group reported the maximum ALT liver enzyme of 88.69 U/L, followed by the coconut and soybean groups, 75.29 U/L and 55.02 U/L, respectively. The olive oil group detected a similar value to the sunflower oil group, at 41.20 U/L and 45.95 U/L, respectively. On the other hand, the flaxseed group presented the lowest reduction in ALT liver enzyme of 33.80 U/L compared to the control group, 33.85 U/L, (Fig 6).

The palm group reported the maximum AST liver enzyme of 92.45 U/L, followed by the coconut and soybean groups, 85.46 U/L and 78.25 U/L, respectively. The olive oil group detected a similar value to the sunflower oil group, at 66.28 U/L and 66.17 U/L, respectively. On the other hand, the flaxseed group presented the lowest reduction in AST liver enzyme of 54.61 U/L compared to the control group, 54.74 U/L.

The palm group reported the maximum ALP liver enzyme of 157.04 U/L, followed by the coconut and soybean groups, 145.22 U/L and 121.55 U/L, respectively. The olive oil group detected a little higher ALP liver enzyme value than the sunflower oil group, at 117.04 U/L and 108.24 U/L, respectively. On the other hand, the flaxseed group presented the lowest reduction in ALP liver enzyme of 96.47 U/L.

Falade *et al.* (2017) had the same results for palm oil by studying the possible health consequences of eating thermally oxidized cooking oils, suggesting hepatic stress and possible hepatocellular damage. Rats' ALT and AST levels were markedly elevated by repeatedly heating palm oil as a result of oxidative damage and lipid peroxidation in the liver tissue. On the other hand, diets supplemented with MSFAs as olive oil, showed a better hepatic profile by decreasing ALT levels and enhancing the overall liver histology, indicating a preventive impact against

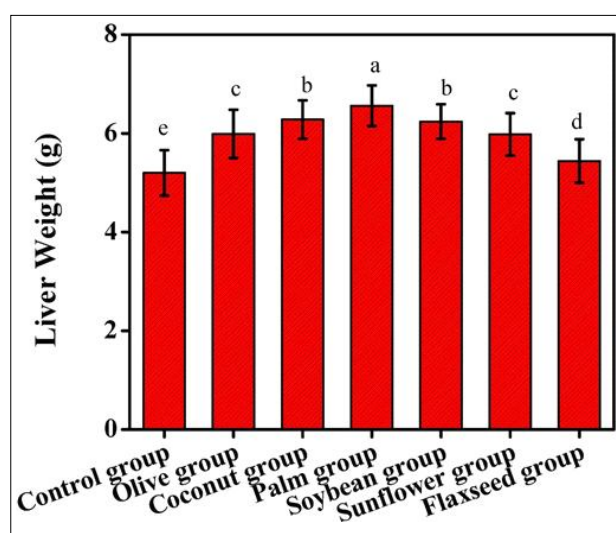


Fig 5: Effect of edible oils on liver weight.

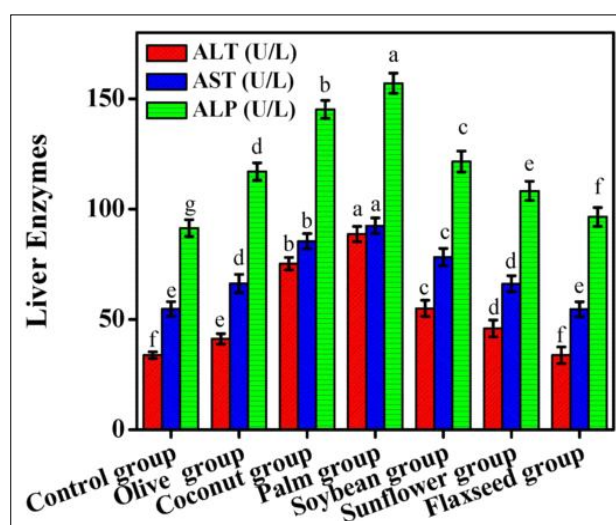


Fig 6: Effect of edible oils on liver enzyme.

inflammation and steatosis by Poudyal *et al.* (2010). In the same manner, the flaxseed group experienced the greatest ALT reduction, followed by the sunflower, coconut and olive oil groups with a reduction in AST levels by the olive and coconut groups by Rabail *et al.* (2024). When compared to the coconut group, the ALT and AST enzyme activities significantly increased with the corn oil intake by Hilakivi-Clarke *et al.* (1997). The liver function results were consistent with Salem (2015), who observed that the olive and thyme oils, as well as the AST, ALT and ALP, had the greatest decrease in these parameters as compared to the control group. It has been demonstrated that PUFA sources, such as fish oil, can mitigate liver enzyme increases by lowering oxidative stress and hepatic triglyceride structure and reducing levels of ALT and AST by Lashin *et al.* (2020). Furthermore, cholestasis, a disorder characterized by the accumulation of cholesterol and bile acids in the liver, has been connected to elevated ALP levels by Alim *et al.* (2024). However, the consumption of soybean oil may make liver damage worse. In rats given a high-fat diet supplemented with soybean oil, elevated levels of ALT and AST were observed, which were correlated with indicators of insulin resistance and inflammation by Deol *et al.* (2015). Jeong *et al.* (2023) showed that feeding obese mice with black and yellow soybeans as a basal diet significantly decreased ALT levels. Additionally, the study found that hepatic genes linked to lipid synthesis were down-regulated, indicating better liver function. Furthermore, the oxidized soybean oil that maternal exposure caused oxidative stress, inflammation and alterations in the liver histopathology of both the dams and their pups. These negative consequences were associated with changes in gene expression relating to lipid production pathways and disruptions in hepatic fatty acid metabolism by Gao *et al.* (2023). Alim *et al.* (2024) studied the consumption of rice bran, olive, soybean and cod liver oils, which dramatically decreased ALT levels, suggesting possible hepatoprotective benefits. These results emphasize the significance of oil type in regulating hepatic health and the varying effects of edible oils on liver enzyme responses.

#### Liver oxidative stress markers

Organisms use antioxidant defense mechanisms to shield themselves from the damaging effects of free radicals. There are both enzymatic and nonenzymatic antioxidants in the antioxidant system. In obese rat models, recent studies have demonstrated the substantial impact of different edible oils on hepatic oxidative stress indicators, particularly MDA, SOD and CAT.

High-fat diet-induced obesity is linked to increased oxidative stress, which is manifested by decreased antioxidant enzyme activities and raised MDA levels. It is believed that one element of the pathophysiology carried on obesity is lipid peroxidation by Pingali *et al.* (2020). MDA, an oxidation product of both arachidonic and linoleic acids, is typically utilized as a marker of lipid peroxidation in oxidative stress. Increased lipid peroxidation causes

the enzymes to become inactive through cross-linking with MDA, which increases the formation of SOD,  $H_2O_2$  and hydroxyl radicals and may further accelerate lipid peroxidation by Lima *et al.* (2022).

The coconut group reported the maximum MDA content of 4.55 nmol/g, followed by the olive and sunflower groups, 4.21 nmol/g and 3.18 nmol/g, respectively. The sunflower oil group detected a similar value to the control group as 2.44 nmol/g and 2.23 nmol/g, respectively. On the other hand, the flaxseed and olive groups presented the lowest reduction in MDA contents of 1.35 nmol/g and 1.58 nmol/g, respectively, (Fig 7).

According to a recent study by Pingali *et al.* (2020), diabetics' lipid profile parameters and cardiovascular parameters, as MDA contents, were significantly improved after the consumption of fish oil supplements regularly. n-3 fatty acid supplementation affects MDA levels as n-3 PUFAs may lower MDA levels by Lima *et al.* (2022).

SOD is the rapid step in the enzymatic system. Mammals' main antioxidant enzyme is SOD, which transforms superoxide anion into hydrogen peroxide, glutathione peroxidase and catalase, which turn hydrogen peroxide into water by Atefi *et al.* (2018).

The flaxseed group reported the maximum SOD activity of 4.68 U/mg followed by olive and sunflower groups at 4.28 U/mg and 3.59 U/mg, respectively. The soybean oil group detected a similar value to the control group, at 3.14 U/mg and 3.25 U/mg, respectively. On the other hand, the palm and coconut groups presented the lowest reduction in SOD activity of 2.76 U/mg and 1.88 U/mg, respectively.

When the hydrogen peroxide concentration is extremely high, catalase is activated. The oxygen consumption affects the change in CAT enzyme activity by Szypulska-Kozarska *et al.* (2019). The flaxseed group reported the maximum CAT activity of 9.66 U/mg, followed by the olive and sunflower groups, 8.41 U/mg and 7.51 U/mg, respectively. The palm oil group detected a similar value to the control group, at 6.51 U/mg

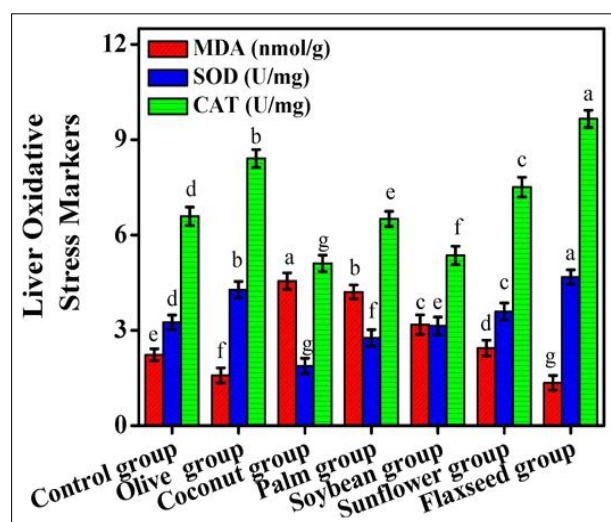


Fig 7: Effect of edible oils on liver oxidative stress markers.

and 6.59 U/mg, respectively. On the other hand, the soybean and coconut groups presented the lowest reduction in CAT activity of 5.36 U/mg and 5.11 U/mg, respectively.

It has been demonstrated that olive oil reduced oxidative stress in the liver by decreasing MDA concentrations and controlling the antioxidant enzyme activities, such as SOD and CAT to reach normal levels, suggesting that it protects against oxidative liver damage by Nakbi *et al.* (2010). Supplementing diabetic rats in coconut oil group showed antioxidant effects that were unique to their tissues with raising the reduced glutathione levels and changing the liver's SOD and CAT activities, indicating a complex function in reducing oxidative stress linked to metabolic diseases by Ströher *et al.* (2019). Since soybean oil is known to be high in PUFAs, which can affect oxidative status, there was not much contemporary research specifically examining its effects on hepatic oxidative stress indicators in obese rats. However, oxidized oils can worsen oxidative stress and the consequences might differ according to how the oil is processed and stored by Suk *et al.* (2023). Concerns about oxidative stability have been linked to sunflower oil because of its high linoleic acid content. Although there was little recent research directly examining its effects on liver oxidative indicators in obese rats, the oil's oxidation susceptibility raises the possibility of elevated oxidative stress in the case of consumption in oxidized forms by Shahidi and Zhong (2010). Haggag *et al.* (2014) also showed that broilers fed sunflower oil, which has the highest PUFA content of any lipid studied, had increased MDA, while the group fed olive oil had extremely little MDA content. In conclusion, the type and quantity of dietary oils that were consumed can have a substantial impact on the liver oxidative stress indicators in obese rats. The effects of other oils, such as soybean and sunflower, may vary depending on their oxidative state and processing techniques. Oils high in antioxidants such as olive and flaxseed oils, tend to strengthen antioxidant defenses and reduce lipid peroxidation.

## CONCLUSION

This study revealed that various edible oils have distinct impacts on reproductive performances and liver functions in hyperlipidemic female Albino Wistar rats. Flaxseed and olive oils demonstrated the most favorable effects by improving reproductive parameters, enhancing antioxidant enzyme activities and reducing oxidative stress. In contrast, palm oil and coconut oil contributed to increased oxidative damage and lower fertility rates, while soybean oil showed moderate effects on both liver enzymes and reproductive performance. These findings suggest that flaxseed and olive oils offer protective benefits under metabolic stress, while the effects of other oils depend on their composition and oxidative stability. The long-term impacts and underlying processes of various edible oils on liver function and reproductive health require more investigation, especially in light of metabolic diseases like hyperlipidemia.

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## Availability of data and materials

Data is provided within the manuscript files.

## Funding

Deanship of Graduate Studies and Scientific Research, Taif University.

## Informed consent

All experimental procedures were approved by the Gulf Countries Association of Sciences in Experimentation on Animals, granted the Ethics Committee approval number 8901/3199 in 2025.

## Conflict of interest

The authors declare no conflict of interest.

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