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Role of Lentils and Olive Oil in Lowering Side Effects of Immunosuppressant Drug in Experimental Rats

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ABSTRACT

The present study was aimed to examine the advantages of lentils and olive oil potential to stimulate immunity and reduce side effects of immunosuppressive drugs in rats. The study involved twenty-five Sprague-Dawley male albino rats, weight (180 ± 10g). The experiment persisted for 60 days (30 days for injured and 30 days for treatment). The rats were randomly allocated into five groups (5 each). The first group served as a negative control group received only a basic standard diet. While twenty rats were orally gavaged with a Cyclosporin A (CsA) (5 mg/kg b.w) for 30 days to induce immunodeficiency and they were reassigned to a positive control group that received the basal diet and three treated groups with 10% lentils, 5% olive oil and other mixture for (10% lentils with 5% olive oil) from basal diet. The treatment duration was 30 days. The results of food analysis demonstrated that the lentils and olive oil rich of many nutrients and bioactive compounds with antioxidant activity. The biological results on all immunosuppressed rat groups treated by lentils and olive oil showed a significant improvement in nutritional indicators (weight gain, food intake and feed efficiency ratio).while the biochemical results in serum showed a significant decrease in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein and total Bilirubin (T.BiL), creatinine, urea, cholesterol, low-density lipoprotein cholesterol (LDLc), very-low-density lipoprotein cholesterol (VLDLc), anti-inflammatory Interleukin-6 (IL-6),Tumor necrosis factor (TNF-α): and C-Reactive protein (CRP) and a significant increase in high-density lipoprotein cholesterol (HDLc) , antioxidant enzymes Catalase (CAT), Superoxide dismutase (SOD), glutathione reduced (GSH) , glutathione peroxides (GPx) and glutathione -s- transferees (GST) and levels of IgE and IgG were compared to the positive control group. The results demonstrated the effectiveness of foods in enhancing immunity and reducing side effects of cyclosporine A (CsA). It can be recommended that patients treating immunodeficiency drug, should consume lentils and olive oil, to improving liver and kidney function, reducing inflammation, enhancing immunity and health.

Keywords: immunity, cyclosporine A (CsA), functional, legumes and oils, rats

1. Introduction

Immunity can be described as the body's ability to resist diseases through various defense mechanisms, and there is adaptive immunity that relies on lymphocytes and produces specific antibodies and develops over time that works to protect the body from pathogens (Lindsay, 2016 and Mohammad *et al.,* 2020). In some cases, immunosuppressant is medically necessary to prevent organ transplant rejection, autoimmune diseases or control inflammatory conditions. The Immunosuppressive drugs reduce immune activity, increase susceptibility to infections and malignancy (Lee *et al*., 2019 and Gonzalez *et al*., 2020).

One immunosuppressive drug is cyclosporin A (CsA) which has been shown to be effective in preventing transplant organ rejection through its ability to suppress T-cell activation. The effectiveness of this drug depends on appropriate dose, timing and duration of treatment (Harris *et al*., 2021).

The concept of functional foods is their ability to promote health and reduce the risk of disease. The functional foods can stimulate immune cells and enhance resistance to infections and diseases, where it

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contains the elements, especially antioxidants, which indicate potential benefits for enhancing immunity and resisting diseases from their deficiency (Gai *et al*., 2022 and Ghafoor *et al*., 2023).

Lentils are rich in comparative phenolic compounds making them a valuable source of bioactive ingredients, ideal for developing functional foods. The highest phenolics and antioxidant activity in peels compared to whole black lentils. Lentils are an important leguminous crop and a staple in many developing countries (Pasqualone *et al*., 2021 and Sidhu *et al*., 2022). Lentils have health-promoting benefits, as anti-inflammatory and antimicrobial properties and rich in proteins, minerals and fiber, which contribute to their nutritional value. The antioxidant properties of lentils are attributed to their phenolic content, which helps reduce inflammation and oxidative stress. In addition, lentils have shown antimicrobial effects, several processing techniques are available to improve the nutritional accessibility of lentils by reducing the effects of certain bioactive compounds (Ciudad-Mulero *et al*., 2020; Yeo & Shahidi, 2020 and Sharma *et al*., 2024).

Olive oil is distinguished by its rich composition and health-promoting properties, as it contains high levels of monounsaturated fatty acids, with phenolic compounds and tocopherols (Vit E). These components contain antioxidant properties that reduce oxidative stress, anti-inflammatory, and provide protection against diseases. (Warhrburg *et al*., 2002 and Al-Talhawi *et al*., 2006). The Olive oil is very important in human nutrition as a preventive agent against many diseases, and protects body tissues from oxidative changes due to free radicals, to contain of polyphenols are beneficial to health, because their antioxidant activity linked with a lower risk of heart disease, and inflammatory conditions. (Miranda *et al*., 2016, Amatori *et al*., 2017 and Nigg *et al*., 2017). Accordingly, this study aimed to assess the possible effects of lentils and olive oil in mitigating side effect of immunosuppressive drugs in rats.

2. Materials and Methods

2.1. Materials

Immunosuppressive drug: Cyclosporine A (CsA) has various trade names, and the name neoral 100 mg Novartis Company capsule was purchased from Egyptian pharmacies city of Mansoura. Drug human therapeutic dose was 100 mg daily which was transformed to animal dose (5mg/kg) in line with previous studies as that documented by Paget and Barnes, (1964). Kits and medical tools for blood analysis were purchased from the Gamma Trade Co., for Pharmaceutical and Chemical, Dokki, Egypt.

Target foods such as lentils (*Lens culinaris* L) and olive oil (*Olea europaea* L) were procured from the Agriculture Research Center, Giza, Egypt.

Experimental rats: twenty-five male albino rats Sprague Dawley strain, weighing (180±10g) were procured from center in faculty of pharmacy, city of Mansoura, Egypt. Basal diet was prepared as per NRC (1995).

2.2. Methods:

2.2.1. Preparation of Lentils powder

Lentils was inspected for impurities, washed separately under running tap water for 1-2 minutes to remove surface contaminants, dirt and unwanted particles. After cooking the lentils in boiling water for half an hour over low heat until fully cooked. Drying process of lentils by distributing the lentils in one thin layer on clean, dry trays under the shade and in a well-ventilated place, and drained and dried in a hot air oven at 50ºC and then ground into fine powder using a laboratory mill. Whole lentils powder was saved in light-protected glass in the refrigerator until used in diet preparation. Experimental diets were prepared by replacing 10% lentils powder 5% olive oil. Taking into consideration their content of protein, fat, and other nutrients, they are deducted from the basal diet. administered for 30 days as treatment (Abdel-Haleem & Omran, 2014 and Kaushik *et al.,* 2014).

2.2.2. Chemical analysis

Total phenolic compounds, flavonoids and antioxidant activity were measured depending on the method of Slinkard and Singleton, (1977), Zhishen *et al.* (1999) and Lasunon *et al*. (2022) respectively.

2.2.3. Induction of immunosuppressant albino rats:

Immunosuppressant rats was induced, administered oral dose of 5 mg/kg. Once daily by stomach tube for 30 days, from freshly prepared of Cyclosporine A (CsA) physiological saline solution according to William *et al*. (1980).

2.3. Experimental animals Protocol:

Twenty-five rats were carefully monitored over a period of five days. to adapt and were fed a basal diet. Five rats served as a negative control group, while twenty rats were orally gavaged with a Cyclosporine A (CsA) (5mg/kg b.w.) for 30 days to induce Immunosuppressant. These rats were then divided into a positive control group (untreated) and three treatment groups: one received 10 % lentils powder, 5% olive oil and the other mixture for 10% lentils with 5% olive oil, of the basal diet components for 30 days. Food and water were provided freely. Food consumption was monitored daily and body weight of rats was measured weekly until the end of the experimental period sixty days (30) days for injuring and 30 days for treatment. All the biological experiments were performed in accordance with globally recognized standards and officially authorized ethical principles of use and care of experimental animals. The study was approved by the Research Ethics Committee, Faculty of Specific Education, Mansoura University.

2.4. Nutritional Parameters:

Food intake, body weight gain, and feed efficiency ratio were calculated as outlined by Chapman *et al*. (1959).

2.5. Biological analyzes*:*

Blood samples were collected after 12 hours of fasting using capillary glass tubes. The blood was then centrifuged at 3,000 rpm for 10 min to separate the serum.

2.6. Determination of some serum biochemical parameters

Serum alanine and aspartate aminotransferase enzymes activity (AST & ALT), alkaline phosphatase (ALP), total protein, total bilirubin, creatinine, urea, total Cholesterol (TC), triglycerides (TG), High density lipoprotein cholesterol (HDLc) were estimated according to the method of Reitman and Frankel (1957), Rec, (1972), Josephson *et al.* (1957), Jendrassik & Grof (1938), Amakasu *et al.,* (2011), Fawcett & Soctt., 1960). N.I.H.P., (1987), Fossati & Prencipe, (1982) and Burstein et al., (1970) respectively. Low density lipoprotein cholesterol and very low density lipoprotein cholesterol determination, calculations were made using the following equation given by Lee and Neiman (1996).

*LDL-c = Total cholesterol –TG / 5 - HDL-c * VLDL-c = TG / 5.

Measurement of some serum antioxidant enzymes as (CAT, SOD, GSH, GPx and GST) activity were measured according to Claiborne (1985), Nishikimi *et al.* (1972), Beutler *et al.* (1963), Paglia & Valentine (1967) and Habig & Jakoby (1974) respectively.

Quantitative analysis of serum inflammatory parameters such as Interleukin-6 (IL-6), Tumor necrosis factor (TNF-α), TNF-a and C-Reactive protein (CRP) were analyzed by the procedure of Grassi *et al*., (1991), Thorell & Lanner, (1973) and Vaishnavi (1993). Immunoglobulin (IgA, IgM, IgE and IgG) were analyzed by Ziva and Pannall (1984).

2.7. Statistical Analysis

All collected data were statistically analyzed using the (SPSS) software, following the methodology outlined by Armitage and Berry (1987). The analysis involved performing an Analysis of Variance (ANOVA), followed by (LSD) test to determine specific group differences.

3. Results and Discussion

Table (1) clear that total phenolic contents, total flavonoids and antioxidant activity contents in lentils and olive oil samples, it was observed that olive oil recorded (139.13 ± 3.96), while lentils was (21.9 ± 31.24). Referring to total flavonoids, it is noted that the highest values were recorded by olive oil (118.53 ± 2.67) while lentils contained the least amount (3.6 ± 0.17). The results for antioxidants

activity showed that olive oil recorded the highest amount (72.20 ± 3.58), whereas lentils recorded the lowest amount (26.94 ± 06.17). It is clear from the previous results that they are consistent with many researches, where it was found lentils are rich in plant chemicals called polyphenols that possess antioxidant properties. Also it was noticed that lentils contain some biologically active phytochemicals such as flavonoids, Extractable and insoluble phenolic compounds (Alshikh *et al.,* 2015 and Bautista-Exp. *et al*., 2018). It was found that the antioxidant activity of the phenols and flavonoids present in olive oil is related to the number of hydroxyl substitutions, amounts of flavonoids, mainly luteolin, apigenin and anthocyanins. The chemical properties of olive oil may enhance its antioxidant properties. Phenols prevent lipid oxidation, and these phenols have been shown to contribute to the stability and antioxidant activity of virgin olive oil (Rice-Evans *et al.,* 1996, Gutierrez *et al.,* 2001 and Ballus *et al.,* 2002). Virgin olive oil has been found to contain at least 30 phenolic compounds, the total amount of phenols in olive oil ranging from 50 to 1000 mg/kg. (Bendini *et al.,* 2007).

Table 1: Total phenolic (TP), total flavonoids (TF) and antioxidant activity (AA) contents in lentils and olive oil samples (mg / 100 g)

Samples / Parameters	Lentils	Olive oil
Total Phenolic (TP)	21.9 ± 31.24 $_b$	139.13 ± 3.96 $_a$
Total Flavonoids (TF)	3.6 ± 0.17$_d$	118.24 ± 2.26$_b$
Antioxidant Activity (AA)	26.94 ± 06.17 $_a$	72.20 ± 3.58 $_c$

Each analysis was performed triplicate. Results were expressed as Mean ± SD in each row having different letters (a, b ,c , d..) are significantly at P>0.05.

3.1. Biological results in experimental rats

3.1.1. Nutritional indicators

Data in table 2 showed: the untreated immunosuppressive rats positive group had a noticeable reduction in body weight gain was observed., and feed efficiency ratio, when compared with negative control group. While, the immunosuppressive rat groups treated with 10% lentils,5% olive oil and their mix led to a significant improvement in body weight gain and feed efficiency ratio (FER), compared to the untreated immunosuppressive group. Despite being significantly lower than the negative control group these parameters were notably higher than in untreated rats. Administration of Cyclosporine A (CsA) is associated with lessening body weight, and associated with muscle wasting and oxidative stress which typically follow continuous exposure to drug, which leads to reduction in body weight and feed efficiency ratio in immunosuppressive group but increased body weight gain and feed efficiency ratio when treated with lentils or olive oil and their mix, despite the consumption of equal amounts of food for all study groups. Also reported that Studied foods, supports the absorption of nutrients and promotes gut health, where a significant portion of the immune system resides. Line with our findings, administrating experimental animals with lentils, olive oil and their mix promoted nutritional indicators, which agrees with Rezvankhah *et al*. (2023) and Sharma *et al.* (2024) accentuate that the lentils are a great source of protein, essential amino acids, minerals and fiber, which contribute to their nutritional value. The antioxidant The beneficial properties of lentils due to their rich phenolic content, which helps reduce inflammation and oxidative stress. Extra virgin olive oil is a natural source of monounsaturated fats, which provide a high caloric density. If added to a diet, it can increase total calorie intake, potentially contributing to body weight gain, however the specific effects of olive oil on body weight in rats (Chaudhary, 2016). The present result was confirmed by Sarmad *et al*. (2019), lentils and olive oil are all nutrient-dense foods that can contribute positively to your overall health and immune system, the efficiency of these treatments in body weight gain and food efficiency ratio, where a potential for incorporating lentils and adding olive oil to your diet strengthens your immune system and supports its ability to infection resistance and reduce inflammation. Their antioxidant, anti-inflammatory, these foods work synergistically to support immune function, reduce oxidative stress, and help maintain long-term health.

Table 2: Nutritional indicators of immunosuppressant rats treated with lentils & olive oil and control

Groups	Variables	Weight gain (gm)	Food intake (gm)	Feed efficiency ratio FER
Control (- ve)		121.13± 13.07 $_{a}$	16.62± 1.04 $_{a}$	0.121± 0.02 $_{a}$
Immunosuppressive rat groups	**Control (+ ve)**	66.79±7.79 $_{e}$	13.45±1.06 $_{b}$	0.082 ± 0.02 $_{e}$
	10% Lentils (L)	110.86±12.77 $_{b}$	17.98±1.80 $_{a}$	0.102 ± 0.01$_{b}$
	5% Olive oil (O)	91.24±10.11 $_{cd}$	16.79 ±1.03 $_{a}$	0.089 ± 0.02 $_{d}$
	10%(L) +5%(O)	102.62 ±11.32 $_{bc}$	17.33 ±1.04 $_{a}$	0.098 ±0.02 $_{c}$

Each value is represented as mean ± SD. Mean values in each column having different letter (a, b, c, d..) are significantly at P>0.05.

According to table (3) the data indicated that immunosuppressive rats "positive control group" were significantly increased liver ALT, AST, ALP, and total bilirubin and decrease in total protein compared to negative control group. Treatment with 10% lentils, 5% olive oil and the other mixture significantly reduced these elevated parameters when compared with the untreated group "positive control " though levels remained higher than control negative group. These results demonstrate the lentils, and olive oil partial protective effects against Cyclosporin A (CsA) drug induced liver damage, suggesting their potential therapeutic value, this is probably attributable to their anti- inflammatory and antioxidant features.

Table 3: Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein and total Bilirubin (T.BiL) levels of immunosuppressive rats treated with lentils, and olive oil compared to control group.

Groups	Variables	ALT (µ/ml)	AST (µ/ml)	ALP (µ/ml)	Total protein (TP) (g/dl)	Total Bilirubin (T.Bil) (g/dl)
Control (- ve)		35.77 ±7.37 $_{d}$	88.50 ±7.55 $_{d}$	77.81 ± 5.14 $_{d}$	7.88 ± 1.19 $_{a}$	0.39 ±0.04 $_{d}$
Immunosuppressive rat groups	**Control (+ ve)**	64.22 ± 7.07 $_{a}$	133.64 ± 9.22 $_{a}$	182.38 ± 7.13 $_{a}$	6.64 ±0.91 $_{ab}$	0.98 ± 0.25 $_{a}$
	10% Lentils (L)	56.24 ± 6.09 $_{ab}$	112.13 ± 9.22 $_{b}$	116.72 ± 7.11 $_{bc}$	7.79 ± 0.98 $_{a}$	0.59 ± 0.09 $_{bc}$
	5% Olive oil (O)	41.27 ± 7.02 $_{c}$	95.14 ± 7.41 $_{c}$	122.18 ± 7.08 $_{b}$	6.61 ± 1.34$_{ab}$	0.62 ± 0.33 $_{bc}$
	10%(L) +5%(O)	41.76 ± 6.78 $_{c}$	94.64 ± 8.25 $_{c}$	112.19 ± 7.37 $_{bc}$	7.01 ± 0.74 $_{ab}$	0.52 ± 0.11 $_{c}$

Each value is represented as mean ± SD. Mean values in each column having different letter (a, b, c, d..) are significantly at P>0.05.

Cyclosporin A (CsA) drug exposure elevates liver enzymes (AST, ALT) by inducing oxidative stress, cellular damage, and metabolic disruption, leading to hepatocyte necrosis and enzyme leakage. This toxicity further exacerbates tissue injury through free radical production, lipid per-oxidation, and antioxidant depletion, causing liver damage our findings are in line with data of Anka and Anita, (2021) & Djohan *et al*., (2021) clear that the supplemented with a combination of lentils and olive oil showed enhanced antioxidant status, improved liver enzyme profiles, and reduced inflammation. The combination of these foods helped protect against liver damage and boosted immune responses. Also it was indicated that lentils and olive oil and their combinations have beneficial effects on liver function and immunity. These foods' antioxidant, anti-inflammatory, and immunomodulatory properties

contribute to improved liver health and enhanced immune responses in rats. Abd El-Ghany (2006) and Albrahim *et al*. (2022) clarify that the olive oil supplementation in rats has shown to improve liver enzyme levels and reduce fatty liver infiltration due to its antioxidant properties. The polyphenols in olive oil, such as hydroxytyrosol, contribute to these beneficial effects. Olive oil has also been demonstrated to modulate immune responses in rats by reducing inflammation and enhancing the activity of immune cells Based on statistical analysis after obtaining results of creatinine and urea table (4) illustrated that normal kidney function with the lowest creatinine and urea levels in negative control group, while positive control group which immune suppression leads to significantly higher of creatinine and urea levels, indicating kidney damage, treatment with 10% Lentils and 5% olive oil and its mixture significantly reduced these elevated parameters when compared with positive control group, Lentils led to moderate improvement in some kidney function as creatinine and urea levels. The study results align with existing research Abd El-Ghany *et al.,* (2015) and Kaur and Das, (2019) demonstrating the kidney-protective effects of these dietary components. Because at lentils are rich in polyphenols and antioxidants, which reduces oxidative stress and inflammation, which are factors to kidney dysfunction in immune-compromised states. Also the lentil consumption can lower blood urea nitrogen and creatinine levels, improving overall renal health. Campos-Vega and Loarca-Piña (2018) accentuate that the Lentil- derived bioactive peptides have been shown to block the activity of the angiotensin- converting enzyme), a key player in kidney damage linked to hypertension. This action improves renal filtration and reduces stress on kidneys and decreased urea and creatinine levels in nephrotoxic rat, these findings suggest that lentils have protective effects against kidney dysfunction, likely attributed to their bioactive compounds with antioxidant and antihypertensive properties

Table 4: Some kidney function parameters levels in immunity suppression rat groups consume lentils, and olive oil compared to control group

Groups	Parameters	Creatinine (mg/dl)	Urea (mg/dl)
Control (- ve)		0.57± 0.14 $_{e}$	23.77± 5.32 $_{d}$
Immunosuppressive rat groups	**Control (+ ve)**	1.71± 0.6 $_{a}$	96.44± 8.52 $_{a}$
	10% Lentils (L)	1.16±0.19 $_{b}$	52.17±8.50 $_{c}$
	5% Olive oil (O)	0.85±0.11 $_{c}$	52.45±6.19 $_{c}$
	10%(L) +5%(O)	0.87 ±0.08 $_{cd}$	57.50 ±5.71 $_{b c}$

Each value is represented as mean ± SD. Mean values in each column having different letter (a, b, c, d..) are significantly at P>0.05.

A study conducted by Covas *et al.* (2018) and Jiménez-López *et al.* (2020) demonstrated that diets rich in olive oil improve kidney health by reducing markers of inflammation and oxidative stress in chronic kidney disease patients. Olive oil's fatty acid profile also enhances lipid metabolism, preventing renal tissue damage caused by high-fat diets or toxins. Olive oil is a widely available natural source of monounsaturated fats and polyphenols with known for its anti- inflammatory effects. It has been shown to protect against nephrotoxicity and oxidative damage, particularly in conditions like immune suppression and toxin exposure. Feng *et al.* (2022) understand that olive oil enhances the bioavailability of fat-soluble antioxidants in diets, the cumulative benefits accelerates of polyphenol-rich diets in reducing kidney disease progression by enhancing antioxidant capacity and modulating immune responses.

Table (5) displays the average values for the serum lipid profile found in negative control group, the lowest values were recorded for total cholesterol (T.CH), triglyceride (TG), low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc), known as bad cholesterol and the highest values were for high density lipoprotein cholesterol (HDLc), known as good cholesterol, that indicator normal levels of serum lipids. On the other hand the positive control group showed the highest levels of T.CH, TG, LDLc and VLDLc with a decrease in HDLc, that reflecting the effect of weak immunity and an increase in harmful lipids. While, Lentils and olive oil treatments significantly reduced of total cholesterol (T.CH), triglyceride (TG), LDLc, VLDLc and improved HDLc levels, which indicates their contribution to improving serum lipid profile compared with the positive control,

but it fell short of reaching the level of the negative control. From the previous results, Anderson *et al*., (2017) indicated that the lentils contain dietary fibers that reduce the absorption of bad cholesterol (LDLc) which helps lower bad cholesterol (LDL) levels in the blood and overall cardiovascular risk , can help improve heart and artery health Jenkins *et al*., (2021) clarify that olive oil is packed with monounsaturated fats and powerful antioxidants, which helps lower bad cholesterol (LDLc) levels and increase good cholesterol (HDLc), thus improving cardiovascular health. It was mentioned that olive oil improves heart health and reduce harmful fats and amplified effects on improving lipid profiles and reducing inflammation.

Table 5: Serum total cholesterol (T.CH), triglyceride (TG), high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) in immunity suppression rat groups compared to control group

Groups	Variables	T.CH (mg/dl)	TG (mg/dl)	HDLc (mg/dl)	LDLc (mg/dl)	VLDLc (mg/dl)
Control (- ve)		85.04 ±12.11 $_{d}$	93.39 ±9.43 $_{d}$	64.85 ±7.74 $_{a}$	18.51 ±0.77 $_{d}$	16.10 ±2.16 $_{d}$
Immunosuppressive rat groups	Control (+ ve)	107.27 ± 6.77 $_{a}$	120.50 ± 9.54 $_{a}$	46.12 ± 6.24 $_{d}$	37.05 ±2.93 $_{a}$	24.10 ±2.65 $_{a}$
	10% Lentils (L)	100.41 ± 3.76 $_{b}$	112.25 ± 5.15 $_{b}$	48.11 ± 5.21 $_{cd}$	32.85 ±3.03 $_{b}$	22.45 ±2.24 $_{b}$
	5% Olive oil (O)	93.14 ± 2.59 $_{cd}$	95.39 ± 7.81 $_{cd}$	58.32 ± 2.99 $_{ab}$	26.74 ±0.55 $_{c}$	19.08 ±2.02 $_{c}$
	10%(L) +5%(O)	95.38 ± 5.65 $_{c}$	106.42 ± 6.73 $_{bc}$	55.24 ± 3.12 $_{bc}$	27.26 ±2.67 $_{c}$	20.88 ±2.82 $_{b}$

Each value is represented as mean ± SD. Mean values in each column having different letter (a, b ,c , d..) are significantly at P>0.05.

The antioxidant enzymes (CAT), (SOD), (GSH), (GPx) and (GST) activity in control group and rat groups with immunity suppression as indicated in Table (6) it is noted that negative control exhibited highest levels of all antioxidant enzymes parameters, while positive control group demonstrated a notable decrease in all antioxidant enzymes, that reflecting immunity suppression .While, 10% lentils, 5% olive oil treatments significantly improved antioxidant enzymes levels (CAT, SOD, GSH and GPX) compared to immunity suppression group , though not to normal levels. These findings demonstrate both lentils, and olive oil effects against oxidative stress. The previous results are consistent with Ganesan and Xu, (2017) who stated that high in polyphenols and flavonoids, lentils have been shown to enhance antioxidant defense systems and reduce oxidative damage. Covas (2007) noted that olive oil known for its high content of monounsaturated fats and polyphenols, therefore olive oil plays a role in reduces oxidative stress and inflammation.

Table (7) displays the some serum inflammatory parameters (IL-6), (TNF-a) and (CRP) in control group and groups with immunity suppression. It was noted that the negative control group showed the lowest significant levels of CRP, TNF and significant increase of IL-6 .While control positive group had significantly higher levels of CRP and TNF ,significantly lower IL- 6 compared to all treated rat groups ,where immunity-suppressed rat groups treated with 10% lentils, 5% olive oil and its mixture showed a significant increase in the levels of IL-6 and significantly lower CRP and TNF compared to the positive control group. However, the treatments lentils and olive oil led to significant improvements (i.e., reductions) in CRP, TNF and increase IL-6 levels, indicating a protective effect against immunity inflammation from Cyclosporin A (CsA) drug.

Table 6: Serum antioxidant enzymes Catalase (CAT), Superoxide dismutase (SOD), glutathione reduced (GSH), glutathione peroxides (GPx) and glutathione -s- transferees (GST) activity in control group and rat groups with immunity suppression.

Groups	Variables	CAT (U/L)	SOD (U/L)	GSH (µmol /L)	GPx (mU/ml)	GST (U/L)
	Control (- ve)	105.04 ±2.09 $_a$	45.12 ±0.22 $_a$	1.93 ±0.19 $_a$	83.72 ±2.32 $_a$	169.59 ±5.11 $_a$
Immunosuppressive rat groups	Control (+ ve)	54.86 ±1.69 $_e$	17.95 ±0.13 $_e$	0.83 ±0.47 $_d$	31.36 ±2.55 $_e$	86.24 ±3.02 $_e$
	10% Lentils (L)	80.33 ±1.23 $_c$	29.65 ±0.23 $_d$	1.03 ±0.18 $_c$	52.37 ±1.03 $_{cd}$	123.97 ±4.01 $_d$
	5% Olive oil (O)	88.63 ±1.38 $_b$	39.54 ±0.73 $_b$	1.47 ±0.73 $_b$	61.28 ±1.93 $_b$	154.31 ±4.17 $_b$
	10%(L) +5%(O)	79.73 ±1.14 $_{cd}$	34.58 ±0.43 $_c$	1.41 ±0.11 $_{bc}$	54.68 ±1.73 $_c$	139.76 ±5.08 $_c$

Each value is represented as mean ± SD. Mean values in each column having different letter (a, b,c , d..) are significantly at P>0.05.

Table 7: Some serum inflammatory parameters Interleukin-6 (IL-6), Tumor necrosis factor (TNF-α): TNF-a and C-Reactive protein (CRP) in control group and groups with immunity suppression at the end of study .

Groups	Variables	IL-6 (pg/ml)	TNF (pg/ml)	CRP (mg/ml)
	Control (- ve)	15.25 ±5.09 $_a$	25.73 ±2.67 $_d$	1.22 ±0.19 $_d$
Immunosuppressive rat groups	Control (+ ve)	9.77 ±1.99 $_d$	35.88 ±6.33 $_a$	2.87 ±0.55 $_a$
	10% Lentils (L)	11.27 ±3.42 $_c$	32.96 ±3.46 $_b$	1.71 ±0.20 $_c$
	5% Olive oil (O)	11.42 ±4.11 $_b$	28.68 ±2.44 $_{bc}$	1.96 ±0.19 $_b$
	10%(L) +5%(O)	11.53 ±3.09 $_{bc}$	30.03 ±2.37 $_b$	1.78 ±0.21 $_{bc}$

Each value is represented as mean ± SD. Mean values in each column having different letter (a, b, c, d..) are significantly at P>0.05.

The study supports the potential of lentils and olive oil in reducing inflammation, particularly in conditions of immune suppression. This aligns with the body of research highlighting the anti-inflammatory benefits of these foods. Zhang *et al.,* (2015) and Guo and Bravo, (2018) accentuate that lentils are rich in polyphenols and bioactive compounds that exhibit anti-inflammatory effects by modulating cytokines such as TNF and IL-6, their role in reducing CRP the linked to their antioxidant and fiber content. Dietary polyphenols influence cytokines, including TNF-α and IL-6, and their potential to reduce systemic inflammation. The fiber content of lentils and its role in promoting gut health and reducing inflammatory markers like (CRP). And also bioactive compounds in legumes, including lentils, and their impact on oxidative stress and inflammation, also the role of lentils as a source of dietary fiber and their impact on inflammatory markers. Sanchez *et al*. (2017) emphasized the importance of following a Mediterranean-style diet leads to a significant reduction in

inflammatory biomarkers. This diet emphasizes consumption of plant-based foods, including fruits, vegetables, whole grains, nuts, seeds, and olive oil.

Table (8) shows that the positive control group had significantly lower values of Immune globulins (IgA, IgM, IgE and IgG) compared to negative control group. While immunosuppressive rat groups witch feeding of 10% lentils, 5% olive oil and the its mixture for 10% lentils with 5% olive oil shows highly significant in all Immune globulins parameters, which proves that treatment with lentils and olive oil improve serum immune globulin levels compared to the positive control group. The results in the table highlight the immunomodulatory effects of lentils and olive oil on serum immune globulins (IgA, IgM, IgE, and IgG) in rats with immunity suppression. These findings align with existing research demonstrating the bioactive properties of these foods. Fareeha *et al*. (2024) and Abdullah *et al*. (2024) and demonstrated that the lentils are rich in phenols, flavones and dietary fiber and are a good source of protein. The lentils enhance macrophage activity, antibody production, cytokine balance, and other antioxidant and anti- inflammatory effects, which are attributed to many health benefits that lead to strengthening immunity.

Table 8: Serum Immune globulins (IgA , IgM , IgE and IgG) in negative control group and rat groups with immunity suppression at the end of study

Groups	Variables	IgA (mg/dl)	IgM (mg/dl)	IgE (IU/ml)	IgG (mg/dl)
Control (- ve)		51.78 ±8.02 $_{a}$	53.25 ± 5.04 $_{a}$	2.11 ±0.17 $_{a}$	458.94 ±11.31 $_{a}$
Immunosuppressive rat groups	**Control (+ ve)**	37.13 ±5.12 $_{d}$	23.38 ±1.60 $_{e}$	0.66 ±0.28 $_{d}$	354.53 ±24.73 $_{e}$
	10% Lentils (L)	45.28 ±6.07 $_{b}$	27.61 ±2.09 $_{d}$	0.79 ±0.11 $_{bc}$	409.14 ±7.09 $_{d}$
	5% Olive oil (O)	48.80 ±4.02 $_{ab}$	48.49 ±5.11 $_{b}$	1.26 ±0.18 $_{b}$	438.41 ±6.11 $_{b}$
	10%(L) +5%(O)	43.14 ±4.08 $_{bc}$	37.44 ±3.17 $_{c}$	1.03 ±0.17 $_{b}$	423.77 ±4.32 $_{c}$

Each value is represented as mean ± SD. Mean values in each column having different letter (a, b, c, d..) are significantly at P>0.05

Polyphenol properties related to their ability to improve immune function and their positive effects on the intestine, suppressing cytokine production, and mediating immune cells, including natural killer cells and macrophages. Beltrán *et al.* (2013) clarify that olive oil, is widely recognized for its health benefits, primarily attributed to its rich amount of monounsaturated fatty acids (MUFA) and phenolic contents, such as oleuropein. These components play significant roles in reducing inflammation and supporting the immune system. It was noticed that olive oil contains several bioactive phenolic compounds, including oleuropein, hydroxytyrosol, and oleocanthal. These compounds contribute to the oil's antioxidant and anti- inflammatory properties. Oleuropein, a key phenolic compound, has been shown to exert anti- inflammatory effects, potentially by modulating the activity of pro-inflammatory enzymes like cyclooxygenase-2 (COX-2). By inhibiting these enzymes, oleuropein helps reduce inflammation, supporting immune function and mitigating conditions like arthritis and other inflammatory diseases. Schwingshackl and Hoffmann (2014) understand that olive oil is predominantly composed of oleic acid, a monounsaturated fatty acid, which has been shown to reduce inflammation in the body. MUFAs help lower the production of help lower the production of molecules like cytokines and prostaglandins, the reduction of inflammation is key to maintaining a healthy immune and preventing chronic diseases linked to inflammation, such as cardiovascular disease and autoimmune conditions.

4. Conclusion

The results of this study revealed that the benefits of both lentils and olive oil and its mixture to reducing inflammation, enhancing immunity, and improving general health from supports liver and renal health by improving lipid profile, antioxidants enzymes, anti-inflammatory and Immune globulins, reducing oxidative stress. The study also recommended that eating these foods reduces inflammation and supports immunity by enhancing immunoglobulin levels and stimulating the activity of lymphatic tissue associated with the intestine. Therefore, eating these foods should be encouraged and included in food products, for patients treated with immunosuppressive drug, because this increases the bioavailability of some biologically active compounds, which leads to lower side effects of drug and improvement healthy.

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