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**Original Article Research**

# EFFECT OF VIRGIN OLIVE OIL SUPPLEMENTATION ON SOME HEMATOLOGIC AND THYROID HORMONES, LEVELS IN RATS

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***ABSTRACT***

The aim of the present study is to investigate the effect of virgin olive oil on some blood parameters in male Albino rats. Thirty male Sprague Dawley rats, (90-110 g), were used in the present study and were divided into three groups (10 in each), 1st group (control), received basal diet and supplemented with 1ml saline. 2nd and 3rd groups received basal diet, and supplemented daily with 1ml/100gm B.W and 2ml/100 gm B.W of virgin olive oil (VOO), respectively. Blood samples were collected weekly from all rats. Whole blood was obtained for determination of some haematological parameters, while sera were collected for the assay of $T_3$ and $T_4$ hormone.

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## Introduction:

Olive oil is extensively used in Middle East, Europe and other parts of the world as a cooking and seasoning medium. Some reports have indicated decreased cardio-vascular dysfunction in persons using olive oil and the relatively decreased incidence of heart related problems in mediterranean countries compared to others in western Europe which attributes increased use of olive oil by these populations, in daily diet both as a cooking and a seasoning medium (Kok and Kromhout, 2004). Beneficial effects of olive oil on cardiovascular system (Covas, 2007) in humans can be attributed to the presence of phenolic compounds reported by some investigators (Tripoli *et al*., 2005). This edible has also been reported to have constituents that provide protection against reactive oxygen species and lipid peroxidation (Fito *et al.*, 2008) Beneficial effects olive oil in reducing lipid peroxidation and in enhancing cardio- protection have been corroborated by other investigators as well (Arrigo *et al.*, 2008). However, no detailed studies have been reported examining the effect of administration of olive oil on hematologic, metabolic and atherogenic parameters in experimental animals or humans. Hence the present study have explored the effect of this virgin olive oil supplementation on hematologic (Total leucocytic, Lymphocytes, Platelets, Hb, RBC, MCV, MCH and MCHC) as well as serum $T_3$ and $T_4$ levels in rats, after oral gavages of varying volumes of oil for a period of 30 days.

**Material and methods:**

**Olive Oil:**

Olive oil in the present study was obtained from olive (Olea europaea; family Oleaceae), a traditional tree crop of Tarhuna City Farms, Libya. The oil was identified by Dr. Salem M. Abd-Alsadiq. Senior Botanist, Department of Crops and Horticulture - Faculty of Agriculture – Tripoli University. Tripoli-Libya. Olive oil was administered in two doses by gastric tube for 4 weeks: Low dose (1 ml / 100g B.W) olive oil and High dose (2 ml / 100g B.W) olive oil daily for 4 weeks (Nandakumaran *et al.*, 2012).

**Animals:**

The study was conducted in the Animal House of National Research Centre (NRC), Cairo, Egypt. Thirty Adult male rats (Sprague Dawley Strain) weighing between 90-110g were used for the present study. The animals used were randomly selected. All rats were active, apparently healthy and free from abnormalities and disease and housed in commercial cages, equipped with automatic drinkers and feeders, at room temperature maintained at 25 $^{\circ}$C, with alternating 12 hour light 12 hour dark cycle. The rats were kept before the experiment for 10 days under such circumstances for acclimatization.

**Feeding regimen:**

Basal diets were formulated to cover the requirements of rats as recommended in NRC (1977). Composition and proximal chemical analysis of formulated diets is shown in Table (1). Diet were subjected to chemical analysis according to AOAC (2012).

**Experimental design:**

The rats were equally and randomly divided into three groups (10 in each): The first group was considered as control group. The rats received basel diet and supplemented with 1ml saline using gastric tube for 4 weeks. The second and third group recieved basel diet, and supplemented with either 1ml/100gm B.W or 2ml/100 gm B.W of virgin olive oil (VOO), respectively, administered using gastric tube for 4 weeks.

**Blood collection and serum separation**:

Blood samples were collected individually by orbital venus plexus technique under mild ether inhalation anaesthesia. Samples were obtained at the early morning before access to feed and water at the end of every week. Portion of blood samples was collected into heparinized tube for haematological parameters including; Total white blood cells counts ($10^3$/µl), lymphocytes (%), platelets ($10^3$/ml), red blood cells counts ($10^6$/µl), haemoglobin concentration (g/dl), packed cells volume (%), mean corpusler volume (fL), mean corpusler haemoglobin (Pg) and mean courposuler haemoglobin concentration (%), which are measured by Mindray Vet Instrument BC 2800-china 2013. The other portion of blood samples was collected into plain tubes and allowed to coagulate at room temperature and centrifuged at 1000 g for 20 min to obtain sera. The clear, non-haemolysed super- natant sera was quickly collected for each animal and stored at -20 $^{\circ}$C for hormonal assay.

**Hormonal assays:** Serum triiodothyronine (T3) and thyroxine (T4) were determined using the method of Tietz (1995), using Enzyme immunoassay kits provided from BioCheck, Inc. Egypt. Serum triiodothyronine (T3) was of minimum detection limint 0.6 ng/ml, while serum thyroxine (T4) was of minimum detection limint of was 0.6 ng/ml.

**Statistical AnaIysis:**

All data are expressed as Means±SE and statistical analysis according to **Snedecor and Cochran (1980)**. was done using SPSS statistical package. Means were compared by the least significance difference test at 5% level of probability (Two way anova test).

**(Table 1): Fatty acid composition of dietary olive oil**

| Fatty acid | g/100g |
|---|---|
| Palmitic acid | 10.28 |
| Palmitoleic acid | 0.77 |
| Stearic acid | 3.39 |
| Oleic acid | 64.80 |
| Lenoleic acid | 14.34 |
| Lenolenic acid | 0.64 |
| Archidonic acid | 0.74 |
| Gadoleic acid | 0.62 |
| Behenic acid | 2.84 |
| SFA | 17.28 |
| MUFA | 66.20 |
| PUF | 14.99 |

SFA: saturated fatty acids; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid

experimental periods.

**Results:**

The present study investigates that supplementation of two different doses of virgin olive oil (VOO); low dose and high dose on some haematological parameters namely (Total leucocytic counts, Lymphocytes, Platelets, RBC, Hb, PCV, MCV, MCH and MCHC) and thyroid hormones changes developed in male rats. In the present study, supplementation of low dose of virgin olive oil (LVOO) showed a significant decrease in the values of Total leucocytic counts, Lymphocytes, Platelets, during 2nd, 3rd and 4th weeks of the experimental period, when compared to those of the basel diet group (Table 2). Moreover, groups supplemented with high dose of virgin olive oil (HVOO), showed a significant decrease in the values of Total leucocytic counts, Lymphocytes, Platelets, during 2nd, 3rd and 4th weeks of the experimental period, when compared with basel diet group. Furthermore, the present results showed that rats supplemented with HVOO, caused significant decrease in the values of Total leucocytic counts, Lymphocytes, Platelets, during 3rd, and 4th weeks of the experimental period, when compared with rats supplemented with LVOO group.

On the contrary, the recorded values for RBCs counts, hemoglobin (Hb) conc., PCV %, MCH pg/ml, and MCHC % showed significant increase in rats supplemented with LVOO during 3rd and 4th weeks of the experimental period, when compared with basel diet group (Table 2). Moreover; there was significant increase in RBCs count, hemoglobin (Hb) conc., PCV %, MCH pg/ml, and MCHC % of rats supplemented with HVOO during 2nd, 3rd and 4th weeks of the experimental period when compared with the basel diet rats.

Furthermore, the present results showed that rats supplemented with HVOO, caused significant increase in the values of RBCs count, hemoglobin (Hb) conc., PCV %, MCH pg/ml, and MCHC % during 3rd, and 4th weeks of the experimental period, when compared with rats supplemented with LVOO group. Meanwhile; There were no significant difference in another blood indice (MCV) among all groups, as compared to the control group receiving normal diet only along the

Low dose of virgin olive oil (LVOO) significantly increased serum triiodothyronine ($T_3$) levels, during 2nd, 3rd and 4th weeks of the experimental period, when compared with control group (Fig. 1). Moreover, HVOO, lead to significant increase in serum triiodothyronine ($T_3$) levels, during 1st, 2nd, 3rd, and 4th weeks of the experimental period, when compared with basel diet (BD) group (Fig. 1). Furthermore, the present results showed that rats supplemented with HVOO, caused significant increase in serum $T_3$ levels during 1st, 2nd, 3rd, and 4th weeks of the experimental period, when compared with rats supplemented with LVOO group. Also, the values presented in (Fig. 2) showed, significant increase in thyroxine ($T_4$) levels in rats supplemented with either low (LVOO), or high (HVOO) doses of virgin olive oil, during 2nd, 3rd and 4th weeks of the experimental period when compared with the basel diet (BD) group (Fig. 2). Furthermore, the present results showed that rats supplemented with HVOO, caused significant decrease in serum $T_4$ values during 2nd, 3rd, and 4th weeks of the experimental period, when compared with rats supplemented with LVOO group.

**Discussion:**

Present study did not show any harmful effect on the various hematologic parameters in male Albino rats, despite of continuous daily supplementation of either low dose (LVOO) or high dose of virgin olive oil (HVOO) for 4 weeks. The present results showed that rats received doses equivalent to 1ml, and 2ml daily of the virgin olive oil for an average rats weighing 90 - 100 g for a continuous duration of 4 weeks, the result indcated significant increases in RBCs counts, hemoglobin (Hb) conc., PCV %, MCH pg/ml, and MCHC % levels. Meanwhile; showed a significant decrease in the values of Total leucocytic counts, Lymphocytes and Platelets. Data of the present results showed, significant decreases in Total Leucocytic count, Lymphocytes %, platelets counts,in rat supplemented with low or high doses of virgin olive oil as compared with control group values.

Leucocytic count in rats receiving massive amounts of olive oil was lower, in two groups of virgin oil supplemented rats than control rats receiving no oil and this could be considered as another beneficial parameter of virgin olive oil have been acclaimed for its protective effects on several of changes associated with heart disease (Acın *et al.*, 2007).

**Table 2: Effect of olive oil supplementation on some haematological parameters in rats.**

| Groups | Parameters | BD | LVOO | HVOO | LSD |
|---|---|---|---|---|---|
| Total leucocytic ($10^3$/µl) | I | 5.12±0.23 | 5.10±0.16 | 5.07±0.15 | 0.15 |
| | II | 5.20±0.15 | 5.04±0.02 | 5.02±0.15 | |
| | III | 5.26±0.11 | 4.98±0.15 | 4.80±0.02 | |
| | IV | 5.26±0.16 | 4.84±0.04 | 4.67±0.05 | |
| Lymphocyte (%) | I | 57.20±2.00 | 56.40±2.64 | 54.10±1.00 | 4.50 |
| | II | 57.90±1.23 | 53.20±2.52 | 50.90±2.00 | |
| | III | 60.00±2.00 | 52.90±2.65 | 48.30±3.00 | |
| | IV | 60.00±0.70 | 49.80±0.95 | 45.20±2.48 | |
| Platelets ($10^3$/ml) | I | 218.0 ±3.29 | 216.0±2.00 | 215.0±2.60 | 4.00 |
| | II | 222.0± 2.73 | 212.0±1.83 | 209.0±2.34 | |
| | III | 223.0 ±2.12 | 210.0±2.94 | 2.00±205.0 | |
| | IV | 223.0±1.41 | 209.0±2.64 | 203.0±2.00 | |
| RBC ($10^6$/µl) | I | 5.40±0.15 | 5.45±0.17 | 5.50±0.10 | 0.13 |
| | II | 5.45±0.10 | 5.50±0.03 | 5.58±0.02 | |
| | III | 5.50±0.12 | 5.64±0.03 | 5.78±0.01 | |
| | IV | 5.54±0.10 | 5.72±0.08 | 5.85±0.15 | |
| Hb (g/dl) | I | 8.15±0.12 | 8.20±0.12 | 8.28±0.20 | 0.31 |
| | II | 8.20±0.18 | 8.35±0.03 | 0.37±8.55 | |
| | III | 8.65±0.15 | 9.10±0.29 | 0.25±9.45 | |
| | IV | 9.10±0.23 | 9.65±0.15 | 9.98±0.07 | |
| PCV (%) | I | 25.80±1.10 | 25.90±0.41 | 26.15±0.50 | 0.60 |
| | II | 25.70±0.69 | 26.13±0.14 | 26.53±0.07 | |
| | III | 26.00±0.85 | 26.80±0.51 | 27.48±0.36 | |
| | IV | 26.00±0.69 | 27.18±0.20 | 27.81±0.43 | |
| MCV (fL) | I | 69.81±0.21 | 47.52±0.37 | 47.54±0.48 | N.S |
| | II | 69.81±0.09 | 47.52±0.50 | 47.54±0.30 | |
| | III | 69.81±0.79 | 47.52±0.61 | 47.54±0.47 | |
| | IV | 69.81±0.46 | 47.52±0.64 | 47.54±0.98 | |
| MCH (Pg) | I | 15.09±0.43 | 15.04±0.78 | 15.05±0.31 | 0.10 |
| | II | 15.04±0.57 | 15.18±0.15 | 0.56±15.32 | |
| | III | 15.72±0.44 | 16.13±0.47 | 0.36.16.34± | |
| | IV | 16.42±0.24 | 16.87±0.30 | 17.05±0.44 | |
| MCHC (%) | I | 31.58±0.30 | 31.66±0.78 | 31.66±0.33 | 0.30 |
| | II | 31.90±0.46 | 31.94±0.35 | 32.22±0.82 | |
| | III | 33.26±0.82 | 33.95±0.50 | 34.38±0.52 | |
| | IV | 35.00±0.30 | 35.49±0.32 | 35.88±0.28 | |
| ANOVA | | P≤ 0.05 | | | |

**Data indicate mean ± standard error at (p≤ 0.05), N= 10 rats, BD= Control, LVOO= low dose of virgin olive oil, HVOO = High dose of virgin olive oil , I= 1st week, II= 2nd week, III= 3rd week, IV= 4th week, LSD= (Least significant difference). WBC= White blood cells, RBC= Red blood cells, Hb= Haemoblobin, PCV= Packed cell volume, MCV= Mean corposular volume, MCH= Mean corposular haemoblobin, MCHC= Mean corposular haemoblobin concentration.**

This was in accordance with the present observations, where feeding rats on the normal diet supplemented with VOO. The healthy effects of VOO on cardiovascular risk factors have been attributed to its high content of MUFAs, such as oleic acid (Massimo *et al.,* 2009; Nandakumaran *et al.*, 2012 and shama *et al.*, 2015). Oleic acid has been reported to exert beneficial effect on the pathogenesis of vascular disease via protection of HDL-C from oxidation and to induce less monocyte chemotaxis adhesion on exposure to oxidative stress (Tsimikas *et al.*, 1999). The significant reduction in lymphocyte count in blood of two groups of virgin olive oil supplemented rats specially in the group receiving higher dose of the virgin olive oil, this difference in lymphocyte count was statistically more significant. Such reduction in lymphocyte count could be associated with altered or diminished immunity in treated animals. More detailed studies are warranted, using a larger animal population. Curvy-Boaventura *et al.* (2007) demonstrated that olive oil reduced lymphocytes proliferation. Platelet

count in rats receiving massive amounts of olive oil was lower, in two groups of virgin oil supplemented rats than control rats receiving no oil and this could be considered as another beneficial parameter preventing formation of thrombus or plaques in lining of coronary or other blood vessels in the body. It is reasonable to assume that study with a larger animal population could clarify the definitive role of virgin olive oil on the status of various hematologic parameters

Phenolic compounds (PhCs) exhibited also some beneficial effects on PLts (Freedman *et al*., 2001; Violi *et al*., 2002; Singh *et al*., 2006 and De Roos *et al*., 2011). PhCs from olive leaf extract significantly inhibited PLts aggregation in vitro, possibly through their $H_2O_2$ scavenging properties, which may offer a degree of protection from thrombosis and other CVD risk factors (Singh *et al*., 2006 and Perona *et al*., 2006 ).

On the contrary, the results indice significant increases in the values for RBCs counts, hemoglobin (Hb) conc., PCV %, MCH pg/ml, and MCHC % in rats supplemented with either low dose of virgin olive oil (LVOO) high dose of virgin olive oil (HVOO), when compared to control group. Meanwhile; There was no significant difference in another blood indice (MCV) among all groups, as compared to the control group receiving normal diet only along the experimental periods. Moreover, OO with phenolic contents were found to increase RBCs count (Huang and Sumpio, 2009), and erythrocytes, as consequence of its anti-oxidant properties (Covas, 2007), as well as Hb concentration, PCV, MCH, and MCHC (Ashour *et al.*, 2007 and Paiva-Martins *et al.* (2009) demonstrated that olive oil shown significantly protect RBCs from oxidative damage in a dose dependent manner. The report confirm the present results where (HVOO) more effective than (LVOO).

Despite the beneficial effects attributed to oleic acid, it is very likely that other minor components with antioxidant and anti-inflammatory properties, such as Oleuropin and its derivative hydroxytyrosol have the strongest radical scavenging properties among all olive oil PhCs (Oram *et al*., 2004); Indicating that olive oil has a beneficial effect on these parameters.

There is a close relationship between alterations of thyroid hormones status and type of fat diet. Data of present study, showed that the group supplemented with either low dose (LVOO) or high dose of virgin olive oil (HVOO), when compared to control group, significant inecrease in serum $T_3$ and $T_4$ levels, as compared with the control group, receiving normal diet. This might be attributed to minor components with antioxidant and anti-inflammatory properties, such as Oleuropin and its derivative hydroxytyrosol that have the strongest radical scavenging properties among all olive oil PhCs, indicating that olive oil has a beneficial effect to improve the thyroid hormone levels, as compared with normal diet group values. It has been reported that, the type of dietary fat affected the metabolism of thyroid cells in rat and consequently thyroid levels in plasma (Siddhanti *et al*., 1990). Moreover; Nema. (2010), found that olive oil improved thyroid hormones levels. Indicating that olive oil has a beneficial effect on these parameters.

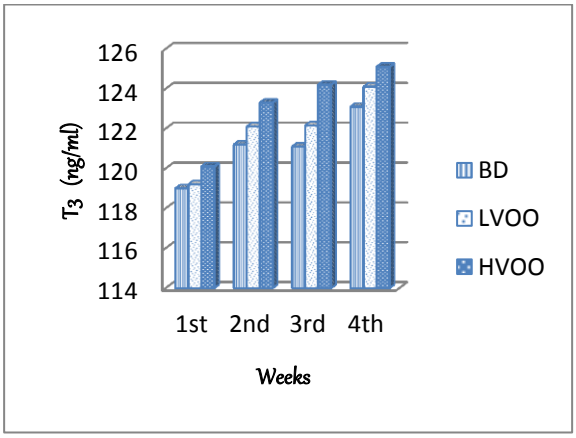


**Fig.1: Effect of olive oil supplementation on serum $T_3$ of rats.** BD= Control, LVOO = low dose of virgin olive oil, HVOO = high dose of virgin olive oil. $T_3$ = Triiodothyronine.

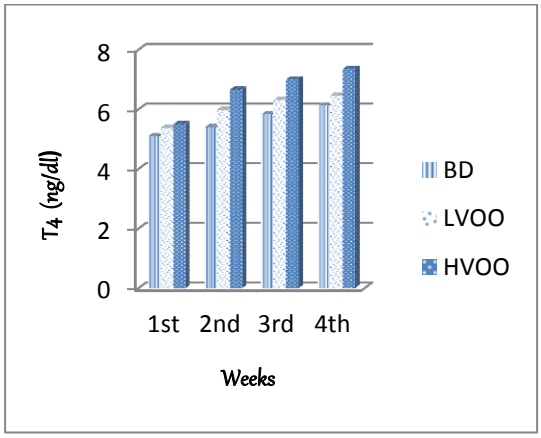


**Fig.2: Effect of olive oil supplementation on serum $T_4$ of rats.** BD= Control, LVOO = low dose of virgin olive oil, HVOO = high dose of virgin olive oil. $T_4$= thyroxine.

**Conclusion**:

The action of both doses of virgin olive oil may be due to the presence of phenolic compounds, oleic acid and linoleic acid, respectively. According to the present data it could be concluded that the antioxidative action of virgin olive oil may be attributed to the antioxidant properties of oleic acid and phenolic compounds that related to their abilities to donate electrons and act as free radical scavengers. Therefore, VOO may be useful in designing dietary strategies to improved hematological picture and thyroid activity.

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