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Research Article

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Effect of Olive Oil Administration on Certain Hematologic and Metabolic Parameters in Pregnant Rats

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ABSTRACT

Objective: Paucity of data on effect of olive oil intake on various hematologic and metabolic parameters in humans or animals have prompted us to assess the effect of oral administration of different doses of this oil on various hematologic and metabolic parameters in pregnant rats.

Material and Methods: Pregnant Sprague Dawley rats were given oral doses of 1 ml, 2 ml and 4 ml olive oil twice per day in divided doses, for 20 days respectively. Control group of pregnant rats were given normal drinking water. Oral feeding of oil was done continuously for the study period and at the end of the study period the animals were lightly anaesthetized with ether and sacrificed to collect blood samples for analysis. Various hematologic parameters such as red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hg), platelets, lymphocytes and mean corpuscular hemoglobin concentration (MCHC) were analyzed by a Hematology Blood Analyzer while metabolic parameters such as cholesterol, triglycerides, urea,uric acid,creatinine and protein were analyzed by specific analytical kits. Activities of antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPX) were assessed by specific analytical kits. Statistical analysis of data was performed using a SPSS data analytical package.

Results: Oral administration of olive oil for 20 continuous days did not significantly alter any of the hematologic parameters studied in Pregnant Rats, compared to control group even when the oil was administered at a relatively massive dose of 4 ml/day. Administration of olive oil appeared to decrease WBC, Hb , platelet and lymphocyte blood concentrations in treated rats , but statistically significant difference (ANOVATest; $p<0.05$) was noted only in the case of platelet concentration. Olive oil administration did not alter the concentrations of protein, cholesterol, urea, triglycerides, uric acid and creatinine in treated groups of rats significantly (Student's t-test, $p>0.05$) compared to those of control rats. SOD level and GPX level in blood of oil- treated animal groups were not significantly different (ANOVA test ; $p>0.05$) compared to control group. Conclusion: We conclude that oral administration of olive oil in pregnant rats, even in massive doses does not cause any significant alterations in hematologic and metabolic parameters and may offer potential benefit although a slight decrease in platelet count was observed which needs further investigation. Further detailed studies are in progress

Key words: *Olive Oil, Hematologic Parameters, Metabolic Parameters, Oral Feeding, Pregnant Rats.*

INTRODUCTION

Olive oil is extensively used in Europe as well as meditaranean countries as a cooking and seasoning medium for many centuries . This oil is established to have a higher content of unsaturated fatty acids and has been widely recommended to be superior to other oils in maintenance of health, although no detailed comparative study has been reported on its efficacy in humans or animals. 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[1,2] compared to others in western Europe

has been attributed to increased use of olive oil by these populations, in daily diet both as a cooking and a seasoning medium. The beneficial effects of this oil on cardiovascular system[3,4] in humans can be attributed to the presence of phenolic compounds reported by some investigators [5,6] This edible oil has also been reported to have constituents that provide protection against reactive oxygen species and lipid peroxidation[7]. Beneficial effects of olive oil in reducing lipid peroxidation and in enhancing cardio-protection have been corroborated by other investigators as well[8]. 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 in humans . Herein we report the effect of this edible oil administration on hematologic (RBC, WBC, Platelets, Hemoglobin, MCV, MCHC) metabolic (cholesterol, triglycerides, protein, urea, uric acid and creatinine) parameters and on status of anti-oxidant enzymes, namely superoxide dismutase and soluble glutathione peroxidase in adult pregnant rats, after oral gavage of varying volumes of oil for a period of 20 days.

MATERIAL AND METHODS

Adult female rats (Sprague Dawley Strain) weighing between 220-240 g were used for the study. The animals used for the study were bred in our Medical Faculty animal house and housed in individual polypropylene cages , at room temperature maintained at 25±1 degree centigrade , with alternating 12 hour light 12 hour dark cycle. Rats were made pregnant using standard laboratory procedure and first day of pregnancy was calculated by noting the presence of sperm in the vaginal plug Body weights of all rats were assessed before the beginning of the study. 3 groups of 5 rats received 0.5 ml, 1 ml and 2 ml of olive oil(RS Brand, Spain) orally twice per day respectively for a continuous period of 20 days . Control group non- pregnant animals were given normal drinking water during the study period. All animals were allowed diet and water ad libitum during the period of study After oral administration of oil for the study period , all animals were weighed and anesthetized lightly with ether and sacrificed for collection of blood samples directly by cardiac puncture. Hematologic parameters such as RBC,WBC, platelets, Hb, lymphocytes and MCHC were determined in blood samples of all study and control groups, using a Hematology Analyzer(ERMA INC, PCE210, Japan) while the concentrations of metabolic parameters namely,protein, cholesterol,triglycerides,creatinine,urea and uric acid in various blood samples were determined using specific analytical kits(Randox Labs,UK).Activity of antioxidant enzymes, superoxide dismutase(SOD), glutathione peroxidase (GPX) in blood samples of study and control groups was determined spectrometrically, using a widely used and specific analytical method (Randox Labs, UK)

Statistical Analysis

All Data are expressed as Means+SEM and statistical analysis was done using SPSS statistical package. Student's t-test or analysis of variance (ANOVA) were used where appropriate. Data were deemed statistically significant, if probability was <0.05

RESULTS

In control group, body weight of rats averaged 227 gm while in the 1ml/day olive oil, 2 ml/day olive oil and 4 ml/day olive oil treated groups, weights of rats averaged 239, 230, and 224 gm before start of experiment After the 20 day study period, the corresponding weights averaged 225 g in control rats and 299 ,296 and 301 gm in corresponding groups of olive oil treated rats. . Table I shows details of some hematological parameters, namely RBC,WBC,Platelets, Hb, lymphocytes and MCHC of the control and treated groups of rats after the 20 day period of oil administration. Although WBC and Hg appeared to be lower in rats receiving higher doses of olive oil, statistical analysis did not show any significant difference ($p>0.05$) between control and treated groups. However, platelets were significantly lower (Student's t-test, $p<0.05$) in rats receiving 1ml and 2 olive oil per day, compared to control although the lower platelet concentration in rats receiving 4 ml oil per day was not significantly different ($p>0.05$) than that of control group. Student's t-test showed that lymphocyte concentration in blood of rats receiving 1ml and 2ml olive oil per day was significantly lower ($p<0.05$) while the difference in lymphocyte

concentration between control rats and rats receiving 4 ml olive oil per day was not statistically significant ($p>0.05$).

Table II shows values of total protein , urea and uric acid in blood samples obtained from control and oil-treated rats after 20 day study period . Statistical analysis did not show any significant difference($p>0.05$) of total protein, urea and uric acid values between control and olive oil treated rats..Total cholesterol averaged 64 ±9 , 69±6 , 62 ±7 and 66±8 mg/dl in control, 1ml, 2ml, 4 ml olive oil per day treated groups respectively. Statistical analysis showed no significant difference ($p>0.05$) in cholesterol level in control and treated groups of rats. Triglyceride values averaged 82±12, 79±9, 77 ±11and 62 ±9 mg/dl in control, 1ml, 2ml, 4 ml olive oil per day treated groups respectively(Fig.1) However, the apparently lower triglyceride values in groups receiving the edible oil were not significantly different from each other (Student's t-test ; $p>0.05$).

The levels of creatinine in blood control and oil treated rat groups are shown in Figure 2. Creatinine concentration averaged 0.60±0.35, 0.90±0.40 , 0.80±0.30 and 0.95±0.35 mg/dl in control, 1ml, 2ml, 4 ml olive oil per day treated groups respectively.Although creatinine values were apparently higher in oil-treated pregnant rats, Student's t-test showed no significant difference in creatinine values between control and study groups($p>0.05$)

SOD Enzyme activity values averaged 1.30±0.18 , 1.48±0.21, 1.50±0.24 and 1.45±0.15 U/ml in control, 1ml, 2ml, 4 ml olive oil per day treated pregnant groups respectively. GPX enzyme activity in blood averaged 1.35±0.18 , 1.52±0.21, 1.56±0.24 and 1.46 ±0.15 Units/L in control, 1ml, 2ml, 4 ml oil treated groups respectively. Although SOD,GPX values appeared to be higher than control group in all oil- treated animals, ANOVA test showed no significant difference ($p>0.05$) between control and treated groups.

Table 1: Haematological parameters in control and olive oil treated Pregnant Rats

	RBC (x 10 6/ul)	WBC (x 10 3/ul)	Platelets (x 10 3/ul)	Hb (g/dl)	MCHC (g/dl)	Lympho (x 10 3/ul)
Control Rats	7.32 ± 0.27	5.35± 0.68	848.0 ± 31.49	12.95 ± 0.34	27.90 ± 0.19	3.32 ± 0.08
Phase I	6.86 ± 2.12	2.60 ± 0.81	515.8 ± 84.98	10.01 ± 2.96	25.05 ± 1.8	2.28 ± 0.43
Phase II	6.21 ± 1.52	2.52 ± 0.62	445.0 ± 71.91	10.50 ± 2.15	28.92 ± 2.47	2.42 ± 0.52
Phase III	7.27 ± 0.32	6.02 ± 0.59	715.2 ± 118.7	12.45 ± 0.57	28.20 ± 0.18	3.70 ± 0.34

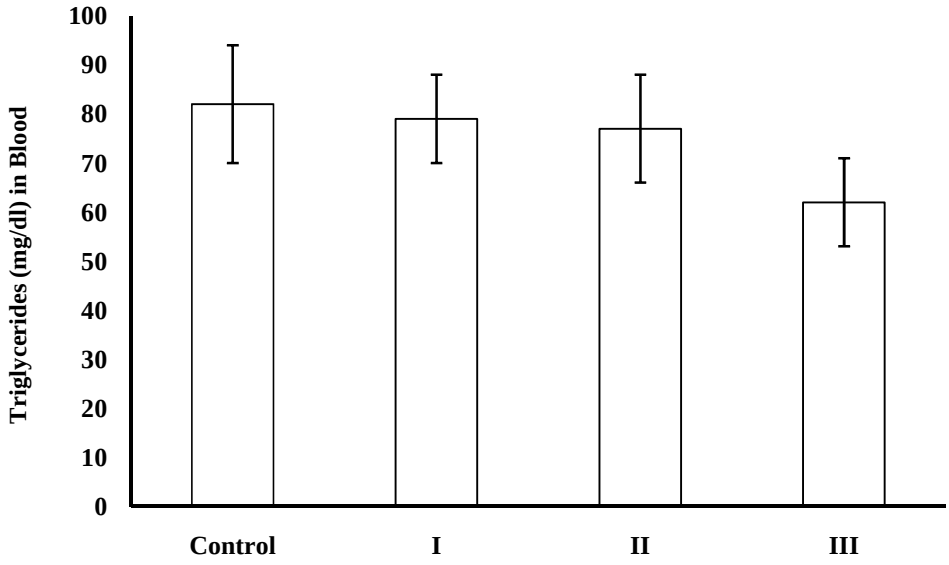
Values are means ± SEM of 5 rats in each group. Statistical analysis was done by ANOVA or Students' t-test where appropriate.
RBC = Red Blood Cell, WBC= White blood cell, Hb= Hemoglobin, MCHC= Mean Corpuscular Hemoglobin Concentration.
Phase I= 1ml dose olive oil/ day
Phase II= 2ml dose olive oil/day
Phase III= 4ml dose olive oil/day

Table 2: Some Metabolic Parameters in control and olive oil treated Female Rats

	Total protein (g/dl)	Urea (mg/dl)	Uric acid (mg/L)
Control Rats	5.99 ± 0.47	40.95 ± 6.82	22.67 ± 0.67
Phase I	5.35 ± 0.25	24.45 ± 6.44	22.52 ± 0.31
Phase II	5.33 ± 0.23	28.20 ± 9.40	22.08 ± 0.55
Phase III	6.06 ± 0.25	30.05 ± 7.72	22.92± 0.21

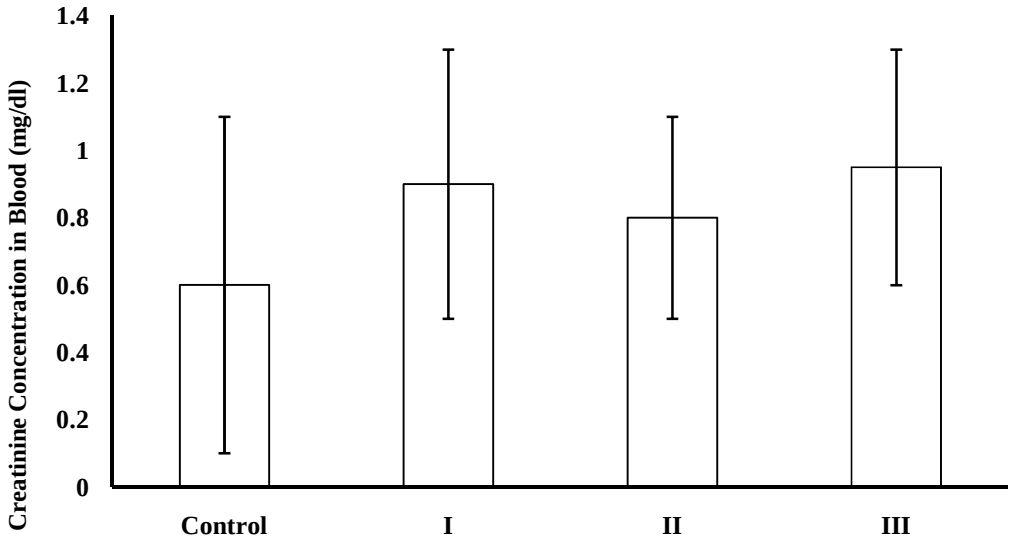
Values are means ± SEM of 5 rats in each group. Statistical analysis was done by ANOVA or Students' t-test where appropriate
Phase I = 1ml dose olive oil/ day
Phase II = 2ml dose olive oil/day
Phase III= 4ml dose olive oil/day

Fig.1: Triglyceride concentrations in blood of control non-pregnant and olive oil treated pregnant rats



Values are Means± SEM of 5 animals in each group ; I=ml/day of olive oil, II=2 ml/day of olive oil ; III=4 ml/day olive oil. Statistical analysis was done by Student's t-test. Control vs I p>0.05 ; Control vs II p>0.05 ; Control Vs III p>0.05

Fig.2: Creatinine concentrations in blood of control non-pregnant and oilve oil treated pregnant rats



Values are Means± SEM of 5 animals in each group ; I=ml/day of olive oil, II=2 ml/day of olive oil ; III=4 ml/day olive oil. Statistical analysis was done by ANOVA test . Control vs I p>0.05 ; Control vs II p>0.05 ; Control Vs III p>0.05

DISCUSSION

Present study did not show any harmful effect of olive oil on the various hematologic and metabolic parameters in pregnant female rats, despite continuous daily administration of graded moderate to high doses of olive for 20 days. Interestingly, when rats received doses equivalent of about 300 ml, 700 ml and 1200 ml daily of the oil comparable to an average pregnant woman weighing 60 kg for a continuous duration of 20 days, the results indicating absence of any significance in hematological or metabolic parameters were surprising and unexpected . In the present study, since olive oil was administered orally, twice daily and continuously for 20 days, we were able to ensure that the animals received the exact dose of the oil during the full course of pregnancy period.

Our data did not indicate any deleterious effect on either cholesterol or triglyceride levels . However, in a study on adult female rats [9] administration of coconut oil in similar high doses , had in fact resulted in lowered cholesterol levels in treated rats. We are unable to explain the reason for the difference in effects of coconut and olive oil on cholesterol disposition in treated rats and further concerted studies are warranted. Surprisingly, even after massive administration of olive oil daily for 20 days, triglyceride, urea, uric acid and and creatinine levels did not increase to significant or abnormal levels, implying that olive oil administration per se does not cause any damaging effects on either the liver or the kidney or the heart. Although in rats receiving higher dose of coconut oil , a relatively more saturated edible oil, treated animals were found to have significantly lower urea level compared to control group in adult female rats [9] but no such effect of olive oil on urea disposition was noted in this study. Absence of significant difference in uric acid and creatinine levels in blood of control and oilive oil treated rats implies that despite receiving massive amounts of the oil for a period of 20 days , olive oil did not cause any major defect in renal function in treated rats.. The absence of hypercholesterolemia and triglyceridemia in olive oil treated rats indicate that even after prolonged administration for a period of 20 days, this edible oil does not affect cholesterol and triglyceride metabolism negatively. Interestingly, platelet count in rats receiving massive amounts of olive oil was lower , in two groups of oil-treated 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.

The significant reduction in lymphocyte count in blood of two groups of olive oil treated rats was surprising though in the group receiving higher amount of 4 ml/day of the oil, this difference in lymphocyte count was not statistically significant. We are unable to attribute the reason or reasons for such an unexpected finding and are unable to ascertain whether such a 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 and we are currently undertaking such a study. We hasten to add that data from experimental animals cannot be extrapolated to humans and hence the same effect may not be present or replicated in humans

In this study, olive oil administration was shown to increase activity of antioxidant enzymes SOD and GPX in all three groups of oil-treated rats though a statistical difference could not be established. The antioxidant function of the above two enzymes , in providing protection from reactive oxygen species is well established. [10,11,12,13] Such a finding of relatively higher anti-oxidant function in animals receiving olive oil is in accord with our earlier findings of increased anti-oxidant activity in rats receiving another edible oil coconut oil in similar experimental conditions [9] as well as in pregnant rats, receiving high amount of coconut oil during pregnancy period [14] We conclude that anti-oxidant activity of phenolic compounds extracted from olive oil [15,16,17] can explain the finding of increased anti-oxidant activity of olive oil in treated animals. We speculate that , by reducing oxidation of LDL moiety mediated through reactive oxygen [18, 19] olive oil could play a beneficial role in preventing formation of plaques and could explain the lower mortality rates from plaque formations ,etc in populations using olive oil as major edible oil, as reported in earlier studies . Anti-cancerous activity of olive oil has been implied by some investigators [20,21]and protective effect of olive oil against helicobacter pylori infection has also been postulated by another research group[22] Possible anti-bacterial role of olive oil in treatment of skin infections in adults has also been reported[23]. We are unable to explain the lower lymphocyte count

observed in the two groups of olive oil treated rats and it is open to speculation whether reduction in lymphocyte count could impact immune function in treated animals. The current studies from our laboratory on rats and other studies from elsewhere [2, 7] strongly point toward absence of any significant noxious effects of consuming olive oil in humans although more detailed studies both in animals as well as in humans are warranted to explore the various effects of olive oil on human and community health.

REFERENCES

1. Kok, F.J. and Kromhout, D. Atherosclerosis: epidemiological studies on the health effects of a Mediterranean diet. *Eur J Nutr*. **43**: 2-5 (2004)
2. Keys, A. Menotti, A. Karvonen, M.J, *et al.* The diet and 15-year death rate in the seven countries study. *Am. J. Epidemiol*. **124**: 903–15(1986)
3. Covas, M.I. Olive oil and the cardiovascular system. *Pharmacol. Res*. **55**: 175–86 (2007)
4. Wahle, K.W. Caruso, D. Ochoa, J.J. *et al*. Olive oil and modulation of cell signaling in disease prevention. Lipids. **39**: 1223-31 (2004)
5. Tripoli, E. Giammanco, M. Tabacchi, G.D. *et al*. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutrition Research Reviews*. **18**: 98–112 (2005)
6. Turner, R. Etienne, N. Alonso, M.G. *et al.* Antioxidant and anti-atherogenic activities of olive oil phenolics. *Int J Vitam Nutr Res* . **75**: 61–70 (2005)
7. Fito, M. Cladellas, M. de la Torre, R. *et al*. Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial. *Eur J Clin Nutr*. **62**: 570-574 (2008)
8. Arrigo, F.G. Cicero, Simona Nascetti, Maria, C. López-Sabater, *et al.* Changes in LDL Fatty Acid Composition as a Response to Olive Oil Treatment Are Inversely Related to Lipid Oxidative Damage: The Eurolive Study. *Journal of the American College of Nutrition.* **27**: 314-320 (2008)
9. Nandakumaran, M. Al-Sarraf, H. Al-Fadhli, R. *et al*. Effect of oral administration of coconut oil on hematologic and metabolic parameters in female adult rats. *Nutr.Ther Metabol.* **27**: 183-188 (2009)
10. Tainer, J.A. Getzoff, E.D. Beem, K.M. *et al.* Determination and analysis of the 2 A-structure of copper, zinc superoxide dismutase *J. Mol. Biol.* **160**: 181–217 (1982)
11. Richardson, J.S. Thomas, K.A. Rubin, B.H. *et al*. Crystal Structure of Bovine Cu,Zn Superoxide Dismutase at 3Å Resolution: Chain Tracing and Metal Ligands *Proc. Natl. Acad. Sci. U.S.A.* **72**: 1349–53 (1975)
12. Epp, O. Ladenstein, R. and Wendel, A. The refined structure of the selenoenzyme glutathione peroxidase at 0.2-nm resolution *Eur. J. Biochem.* **133**: 51–69 (1983)
13. Muller, F.L. Lustgarten, M.S. Jang, Y. *et al*. Trends in oxidative aging theories. *Free Radic. Biol. Med.* **43**: 477–503 (2007)
14. Nandakumaran, M. Elisaveth Angelaki, Nasser Al-Azemi, *et al*. Influence of coconut oil administration on some hematologic and metabolic parameters in pregnant rats. *J. Mat-Fet Neonat Med.* **24**: 1254-1258 (2011)
15. Servili, M. Baldioloi, M. Miniati, E. *et al*. Antooxidant activity of new phenolic compounds extracted from virgin oilive oil and their extraction with alpha-tocopherol and beta-carotene. *Rv. I. Sost. Grasse*. **73**: 55-59 (1996)
16. Visioli, F. and Galli, C. Oleuropein protects low density lipoproteins from oxidation. *Life Sci*.**55**: 1965-1971 (1994)
17. Papadopoulos, G. and Boskou, D. Antioxidant effect of natural phenols in olive oil. *J. Am. Oil chem. soc.* **68**: 669-671 (1991)
18. Giovaninic, C. Strafce, E. Modesti, D. *et al.* Tyrosol, the major olive oil biophenol, protects against oxidized-LDL-induced injury in Caco-2-cells. *J. Nutr*. **129**: 1269-1277 (1999)

19. Baldioli, M. Servili, M. Perretti, G. *et al*. Antioxidant activity of tocopherols and phenolic compounds in virgin olive oil. *J. Am. Oil chem.Soc*. **73**: 1589-1593 (1996)
20. Owen, R.W. Haubner, R. Wurtele, G. *et al*. Olives and olive oil in cancer prevention. *Eur J Cancer Prev*. **13**: 319-26 (2004)
21. Menendez, J.A. and Lupu, R. Mediterranean dietary traditions for the molecular treatment of human cancer: anti-oncogenic actions of the main olive oil's monounsaturated fatty acid oleic acid (18:1n-9). *Curr Pharm Biotechnol*. **7** : 495-502 (2006)
22. Romero, C. Medina, E. Vargas, J. *et al*. *In vitro* activity of olive oil polyphenols against Helicobacter pylori. *J Agric Food Chem.* **7(55)**: 680-6(2007)
23. Verallo-Rowell, V.M. Dillague, K.M. and Syah-Tjundawan, B.S. Novel anti-bacterial and emollient effects of coconut oil and virgin olive oils in adult atopic dermatitis. Dermatitis **19**: 308-315 (2008)