ADVANCES IN BIORESEARCH
Adv. Biores., Vol 8 [3] May 2017: 67-74

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.8.3.6774

Advances in Bioresearch

ORIGINAL ARTICLE

Antioxidant Modulating effect of Olive oil (*Olea europeaea*) in the management of oxidative stress to combat obesity

Farooq Ahmed Wani[1]*, Shaik Rahiman[2], Abdul Rahman Hamdan A Almaeen[3]
[1,3]Department of Pathology, College of Medicine, Aljouf University, Aljouf, Saudi Arabia
[2]Department of Biochemistry, College of Medicine, Aljouf University, Aljouf, Saudi Arabia
Email: rahimhi@gmail.com

ABSTRACT
The current study was aimed to explore Antioxidant Modulating effect of Olive oil (Olea europeaea) in the management of oxidative stress to combat obesity. Swiss albino mice were randomly grouped in five groups (n = 10) to receive a standard diet (Control group), a standard diet and olive oil (OO group; 1.3 ml/kg, b.w./p.o.), a high-fat diet (HFD group) and a high-fat diet supplemented with olive oil (HFD+OO group; 1.3 ml/kg, b.w./p.o.) for 16 weeks. We determined biochemical parameters that included: Lipid profile (TC, TG, LDL-C, HDL-C), glucose, ALP, AST, ALT, antioxidative profile (SOD, CAT, GPx, MAD, Total nitrates,) and total bilurubin in serum. Statistical analysis was done with SPSS for Windows version 17.0. Serum glucose levels was significantly decreased in Olive oil (1.3 mL/kg, b.w./p.o.) treated Groups (OO and HFD+OO) ($p < 0.05$) as compared to high fat diet fed group (HFD) animals. HFD+OO group animals significantly decrease ($p < 0.05$)in levels of serum TAG levels as compared to HFD group. Our results showed significant decrease if serum Total cholesterol (TC), LDL-C, in control, OO and HFD+OO Group animals in comparison with HFD Group ($p < 0.05$). Furthermore, the serum levels of HDL-C was significantly increased ($p < 0.05$) in HFD+OO group animals as compared to HFD group. HFD+OO group mice showed drastically decreased serum AST, ALT and ALP levels as compared to HFD group mice. Serum GG, total bilurubin levels were significantly increased in HFD group as compare to Control group, but when treated with Olive oil it was significantly decreased in HDF+OO group animals. Compared with the control group, the activities of SOD, CAT, GPx in HFD group mice were significantly decreased ($p < 0.05$). Serum total nitrate levels also significantly decreased ($p < 0.05$) in HFD group as compared to other three groups. Serum MDA levels were significantly increased ($p < 0.05$)in HFD group as compared to Control group. The data of the current study provides experimental evidence for the anti-obesity effect of olive oil as an alternative remedy for the prevention as well as in the treatment for metabolic inflammation in diet induced obesity and other metabolic diseases.
Keywords: *Olive oil, Obesity, Antioxidant enzymes, Metabolic syndrome, Lipid profile, Liver enzymes, High fat diet induced obesity*

Received 24.02.2017 Revised 19.03.2017 Accepted 27.04.2017

How to cite this article:
F Ahmed Wani, S Rahiman, A Rahman Hamdan A Almaee. Antioxidant Modulating effect of Olive oil (*Olea europeaea*) in the management of oxidative stress to combat obesity. Adv. Biores., Vol 8 [3] May 2017.67-74

INTRODUCTION

Obesity has emerged as one of the leading public-health issues in the past decades. More than 1.1 billion people are overweight worldwide and 312 million are classified as obese [1]. Obesity is a major health concern, which leads to a reduction in life expectancy of nearly 8 years, and to several comorbidities, including the metabolic syndrome consisting of insulin resistance, type 2 diabetes, cardiovascular disease, fatty liver disease, cancer, hypertension, stroke, dementia, and obstructive sleep apnoea [2, 3]. Epidemiological, clinical, and animal studies have shown that obesity is coupled with altered redox state and increased metabolic risk [4, 5]. Oxidative stress can be a consequence, but also a trigger of obesity. Chronic hypernutrition, high fat high carbohydrate (HFHC) meals, as well as high dietary saturated fatty acids (SFA) and trans-fatty acids, stimulate intracellular pathways, leading to oxidative stress through multiple biochemical mechanisms [11, 37]. Oxidative stress could play a causative role in the development of obesity by stimulating white adipose tissue deposition and altering food intake: cell culture and animal studies show that oxidative stress increases pre-adipocyte proliferation, adipocyte differentiation and size of mature adipocytes [16, 25]. . Liver oxidative stress mainly due to glucose auto

oxidation , shifts in redox balances, decreased concentration of low molecular weight antioxidants in tissue as well as impairment of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT)defense functions [15].

Recently, natural antioxidants gaining popularity in the treatment of obesity to overcome the adverse effects of synthetic anti obesity drugs. The olive tree (*Olea europaea*) is native to the Mediterranean region and has been known for its medicinal properties since ancient times. Olive leaves and their extracts are used for a number of different purposes, such as to provide nutrients, control weight loss and help fighting against a variety of illness. Olive oil has been widely used in traditional medicine for several thousand of years in countries of Mediterranean basin. Coronary heart disease, and colonic and prostate cancers were found to be very low in the Mediterranean countries [17].

Furthermore, it also gain attention by the most of the researchers and practitioners as a alternative therapy for some metaflamations rather than recognized as nutritional food only. Olive oil has been commercialized as a food supplement which can be consumed in the form of, syrup and capsules. Earlier studies reported the potential therapeutic effects of Olive tree such as, antioxidant properties, hypotensive, hypoglycemic, cardiovascular and hepato-protective effects. [9, 22, 24, 34, 10]. Some studies on mammals has been showed that, olive oil has positive effects on regulation of cholesterol and oxidation of bad cholesterol (i.e LDL). Olive oil rich in monounsaturated fatty acids (MUFA) in the form of oleic acid accounts for 70-80% of total fatty acids [26]. Gimeno E *et al,* [13**]** reported that daily consumption of virgin olive oil daily about 2 tablespoons for 1 weeks helps in the lowering of LDL cholesterol oxidation and increase the antioxidants compounds in the blood. It also reported that olive oil maintained the plasma lipid pool by lipid peroxidation and antioxidant parameters regulation [14].

Earlier scientific literature revealed that the potential pharmacological effects of Olive oil has been reported in different pathological conditions such as a traditional anti-obesity drug and anti oxidative remedy in the management of metabolic disease. The aim of the present study to access the effect of Olive oil (Olea europeaea) on anti-oxidative enzymes profile in the management of oxidative stress to combat obesity in an experimental animal model.

MATERIALS AND METHODS

Chemicals

All the chemicals and reagents used in this experiment were research analytical grade from sigma USA. All assay kits were purchased from Biovision, USA. Extra virgin olive oil was purchased from the local market, Sakaka, Aljouf, K.S.A.

Animal diet

Basal diet was purchased from Grain Silos & Flour Mills Organization, Riyadh, K.S.A. It contained the ingredients of 4 % fat; 20% crude protein; 43.5% crude fiber; 0.5% salt, 1% calcium, 0.6% phosphorous, 6% ash, 20 IU/g vitamin A, 20 IU/kg vitamin E 20 IU/g vitamin D, and trace amounts of copper, iron, cobalt, iodine, manganese, zinc and selenium. High-fat diet (HFD) in which 42% of the energy is derived from fat, was prepared by the addition of 1.5% cholesterol (Sigma Aldrich, USA) and 8% coconut oil to the basal diet [30]. The HFD was prepared after every 2 days, stored at 4°C.

Assay Kits

Assay kits for detecting serum glucose, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), Total bilurubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and alkaline phosphatase(AST) were purchased from Biovision, USA **.** Superoxide Dismutase (SOD) , Catalase, Glutathione Peroxidase (GPx), MDA and Total nitrites were purchased form cell bio labs USA.

Acute toxicity

The method described by **Lorke [27]** with slight modification was used to determine the safety of the extra virgin olive oil dose for mice. Briefly, normal healthy mice were divided into groups of five mice in each cage. Olive oil doses of 0.3, 0.6, 0.9, 1.3, and 1.6 ml/kg b.w. were orally administered to animals 10 in number. Free Access to food and water was provided. Toxic symptoms and the general behavior of mice were observed continuously for 1 h after the treatment, intermittently for 4 h and thereafter over a period of 24 h. The mice were further observed for up to 14 days following treatment for any signs of toxicity and mortality.

Animals and experimental design

Adult male Swiss albino mice weighing 20-25 g were obtained from animal house, College of Medicine, Aljouf University. All protocols were approved by the college ethical committee, Aljouf University. All mice were fed *ad libitum* with standard laboratory pellet diet and free access to tap water. The experimental mice were maintained under a constant 12 hour light and dark cycle at room temperature.

Animals were acclimatized to the new experiment environments for 3 days before the study. After that, the animals were divided in to five groups each group 10 in number. Control group (Group I) animals fed with a normal diet (ND) and animals fed with high – fat diet (HFD) termed as high-fat diet group (HFD Group /Group II). One group animals (Group III) received olive oil (1.3 ml/kg b.w orally) alone (olive control group/Positive group). The other two groups animals (Group IV –V) received HF along with olive oil (1.3 ml/kg b.w, orally). All mice were fed for 16 weeks. During every two weeks animals body weight were measured. At the end of the experimental period mice were overnight fastened for 12 hours and followed by diethyl ether anesthetization. Blood samples were collected by animals cervical decapitation and stored at −70∘C until biochemical analysis.

Biochemical analysis

Measurements of lipid profiles and serum glucose

Among lipid profiles parameters, concentration of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), serum glucose, and total bilurubin were measured by enzymatic colorimetric methods using commercial assay kits (Biovision, UK)as per manufacture instruction by using Chemwell Semi Biochemistry Analyzer (USA).

Biochemical serum enzyme estimations

Among serum enzyme profile, Alkaline phosphatase (ALP), alanine aminotransferase (ALT), and alkaline phosphatase (AST) Superoxide Dismutase (SOD) , Catalase, Glutathione Peroxidase (GPx), MDA and Total nitrites were estimated according to the reported methods using assay kits (Cell biolabs, USA) by using Chemwell Semi Biochemistry Analyzer (USA).

Statistical analysis

All assay parameters in this study were run in triplicate. Data are expressed as means ± SD. One –way analysis of variance (ANOVA) was applied by using SPSS, Statistical Package for Social Science, (IBM, SPSS Ver. 17.0,SPSS Company, London, UK).for the statistical significance with post-hoc test. $P < 0.05$ was considered as statically significance.

RESULTS AND DISCUSSION

Our results showed that, the serum glucose levels was significantly decreased in Olive oil (1.3 mL/kg, b.w./p.o.) treated Groups (Control, OO and HFD+OO) ($p < 0.05$) as compared to high fat diet fed group (HFD) animals (Figure 2). In OO-Group animals the serum glucose levels are significantly ($p < 0.05$) increased as compared to control group. The Mediterranean diet with its high content of olive oil represents a health and disease preventive diet and reduces mortality from heart disease. Insulin resistance is associated with a number of metabolic disorders such as obesity, hyperlipidemia, and hypertension. HFD intakes were shown to contribute to syndromes such as hyperlipidemia, glucose intolerance, hypertension, and atherosclerosis. Numerous evidences indicated that in experimental animals, high-fat diets resulted in disturbance in glucose metabolism and impaired glucose tolerance [8], and the present study we observed a significant decrease ($p < 0.05$) in serum glucose levels (115.52 ± 0.99 mg/dl) in HFD+OO group animals after 16 weeks of experimental period as compared to high fat diet fed mice (163.49 ± 0.64 mg/dl) . Our results are supported by the previous studies [19].

The serum lipid levels results are given in Table 1. The TAG levels in HFD Group were significantly increase as compared to control group($p < 0.05$). No significant differences observed between control and olive oil (OO)group animals as well as, high fat diet with olive oil(HFD+OO) group animals significant decrease ($p < 0.05$)in levels of serum TAG levels as compared to HFD group (Figure 1a). Figure 1b,c ,reviled the significant decrease if serum Total cholesterol (TC), LDL-C, in control, OO and HFD+OO Group animals in comparison with HFD Group ($p < 0.05$). Furthermore, the serum levels of HDL-C was significantly increased ($p < 0.05$) in HFD+OO group animals as compared to HFD group (Figure 1d). Previous studies stated that olive oil can induce favourable changes in serum lipid profile and aid in the improvement of endothelial function [33]. Further, dyslipidemia is another important hallmark in the pathogenesis of obesity characterized by hypertriglyceridemia with decreased level of LDL and VLDL [23, 31]. Chronic dyslipidemia has been characterized as a major risk factor for cardiovascular risk, including atherosclerosis [18, 29]. In the present study, mice treated of olive oil showed that decrease serum total cholesterol, serum triglycerides and low density lipoprotein (LDL) levels as compared animal group fed with high-fat diet. These results are in agreement with the previous studies in different animal model fed with high-fat diet and olive oil [20, 32, 6]. The level of serum HDL –cholesterol produced significant increase in treated group animals compared to control and HFD group. This results contradicted with Louka, M.L *et al*., [28], but conceded with Paoli et al. [32] results. This lipid lowering property of olive oil is mainly due to the presence of oleic acid [21].

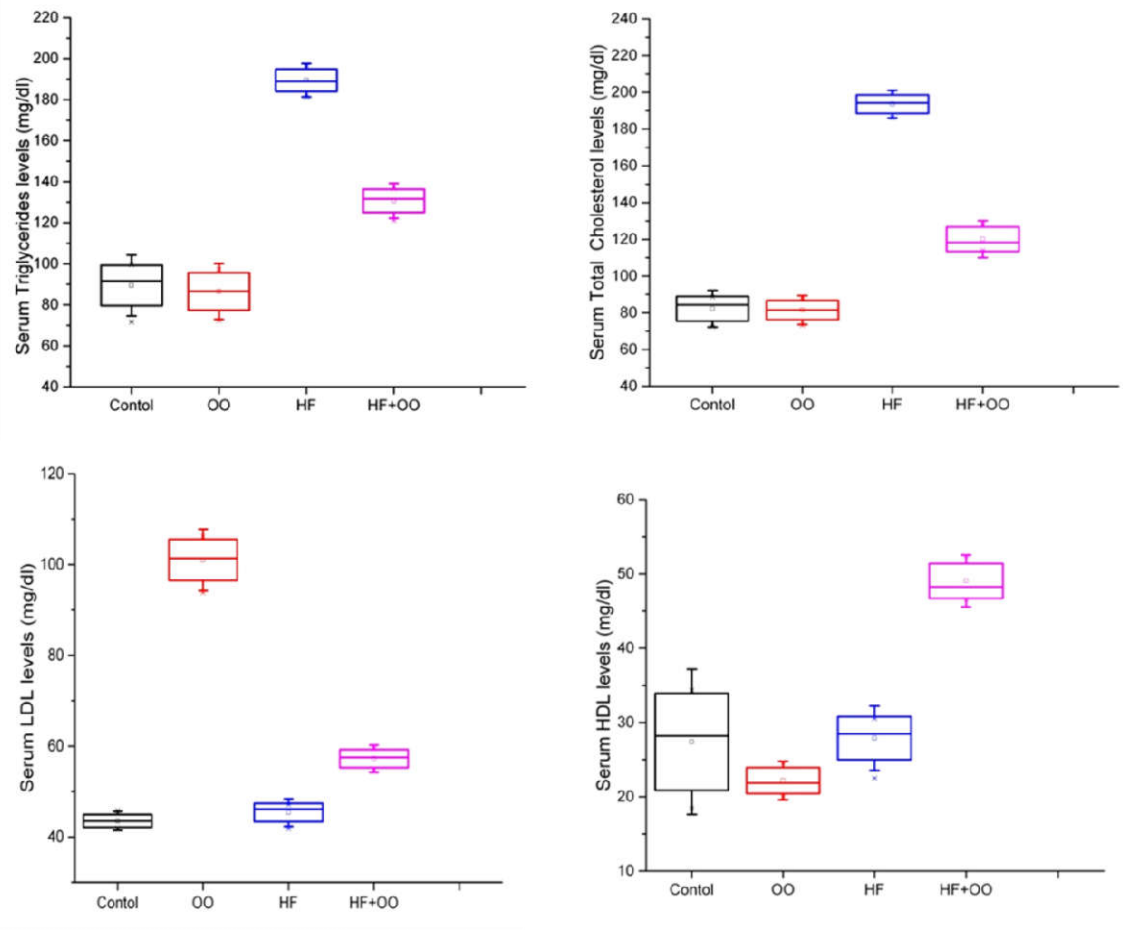

Figure 1a-d: Effect of Olive oil on serum Lipid parameters (TGA, TC, LDL, HDL) in diet induced obesity experimental model.

The serum levels of AST was significantly increased in HFD Group as compared to other groups ($p > 0.05$). HFD+OO group mice showed drastically decreased serum ALT levels as compared to HFD group mice. The levels of ALP in HFD+OO group was significantly decreased as compared to HFD group animals. The studied serum enzyme parameters in olive control group and control groups were revealed no statistical difference (Table 2) whereas, both groups showed statistically significant difference as compared to HFD group animals.. Serum GGT was significantly increased in HFD group as compare to Control group, but when treated with Olive oil it was significantly decreased in OO group and HDF+OO group animals. Serum total bilurubin levels were significantly decrease in HFD+OO group as compared to the HFD group). Serum AST, ALT, ALP and total biluruibin are the enzyme biomarkers to monitor the liver structural integrity and its damage liver toxicity conditions [38, 7]. Generally high-fat diet increases these enzymes through the induction of oxidative stress in the liver [7]. A HFD may increase the synthesis of fatty acids in the liver and the delivery of free fatty acids to the liver. It may also decrease β-oxidation of free fatty acids, which may, in turn, cause fat accumulation in the liver. In our study, olive oil treatment has ameliorated ALT and GGT levels increased by HFD, which was in accordance with the data of Raja *et al*. [35]. This result proved that excessive fat supplementation can show deleterious toxic effect on liver by the production of free radicals and ROS (reactive oxygen species) [7], which is minimized in HFD+OO group mice treated with olive oil. Generally in human population the elevated levels of serum ALT has been suggested as a fatty liver disease [12], but our study results proved that, supplementation of olive oil can decrease the serum ALT levels and protect the liver form toxic effect but it need further scientific trails in human population.

The effect of olive oil on the activities of antioxidant enzymes in serum of HFD induced obese mice were shown in (Table 3). Compared with the control group, the activities of SOD, CAT, GPx in HFD group mice were significantly decreased ($p < 0.05$) (Figure 3a-c). Serum total nitrate levels also significantly decreased ($p < 0.05$) in HFD group as compared to other three groups (Figure 3d) . Serum MDA levels

were significantly increased ($p < 0.05$)in HFD group as compared to Control group (Figure 3e). Antioxidative enzymes, including SOD, GPx and CAT, are regarded as the first line of the antioxidant defence system against ROS generated in vivo during oxidative stress. Their ability to decompose superoxide and peroxide while blocking lipid peroxidation as well as their involvement in cellular defence mechanisms helps to protect tissues against oxidative damage. SOD scavenges the superoxide radical by converting it to hydrogen peroxide and molecular oxygen. CAT is a hemeprotein, which catalyzes the reduction of hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals. GPx, an enzyme with selenium, works together with glutathione (GSH) in the decomposition of hydrogen peroxide (or) other organic hydroperoxides to non-toxic products at the expense of reduced glutathione.

In this study, significant decrease in the activities of SOD, CAT and GPx were observed in serum, HFD diet induced obese mice. The reduced activities of these antioxidant enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxides.. The increased MDA level in the untreated obese group clearly demonstrated that high fat consumption was attributed to increased oxidative stress. Olive oil supplementation was found to improve the endogenous antioxidant defense system by enhancing the antioxidant enzymes activities in vivo. The groups treated with olive oil showed a significant elevation in their SOD, GPx, GSH, and CAT activities compared to the untreated obese rats. These results are aligned with the reduction of MDA levels, which may be related to the ability of AL-H to suppress lipid peroxidation due to having an increase in the activity of antioxidant enzymes, regardless of the availability of lipid substrates [36].

Oxidative stress is greatly increased on the treatment with high fat diet in the form of enhanced lipid peroxidation reactions and depletion of tissue antioxidant like GSH; and higher nitrostative stress, in men [40]. In present study, increased serum total nitrite/nitrate, and decreased GSH levels confirm the role of oxidative and nitrostative stresses. Treatment with olive oil significantly attenuated these HFD induced oxidative/nitrosative stress.

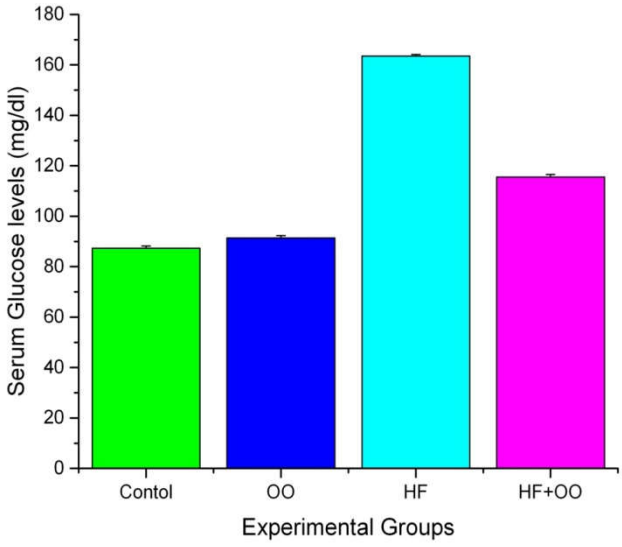

Figure 2: Effect of Olive oil on serum glucose in diet induced obesity experimental model.

Table 1: Hypolipidemic effect of olive oil against diet induced obesity in rats.

	TGA	TC	HDL	LDL
Control	89.44± 9.87	82.21± 6.66	27.39±6.52	43.54±1.39
Olive oil	86.49± 9.11	81.51±5.21	22.17±1.73	101.04±4.51
High fat	189.47± 5.41	193.54±5.01	27.88±2.89	45.37±2.01
High fat + Olive oil	130.65± 5.67	120.05± 6.69	49.04± 2.33	57.23±1.99

All values were expressed in Mean ±SD, all measurement unit are mg/dl

Table 2: Effect of olive oil on Serum enzyme levels in HFD induced obesity model

Experimental groups	AST	ALT	ALP
Control	99.36±9.26	48.96±4.07	131.61±2.15
Olive oil	101.14±2.67	48.87±2.16	134.20±2.96
High fat	146.82±5.05	130.53±2.31	196.69±3.32
High fat + Olive oil	139.77±2.96	49.44±1.65	114.26±4.37

All values were expressed in Mean ±SD, all measurement unit are IU/L

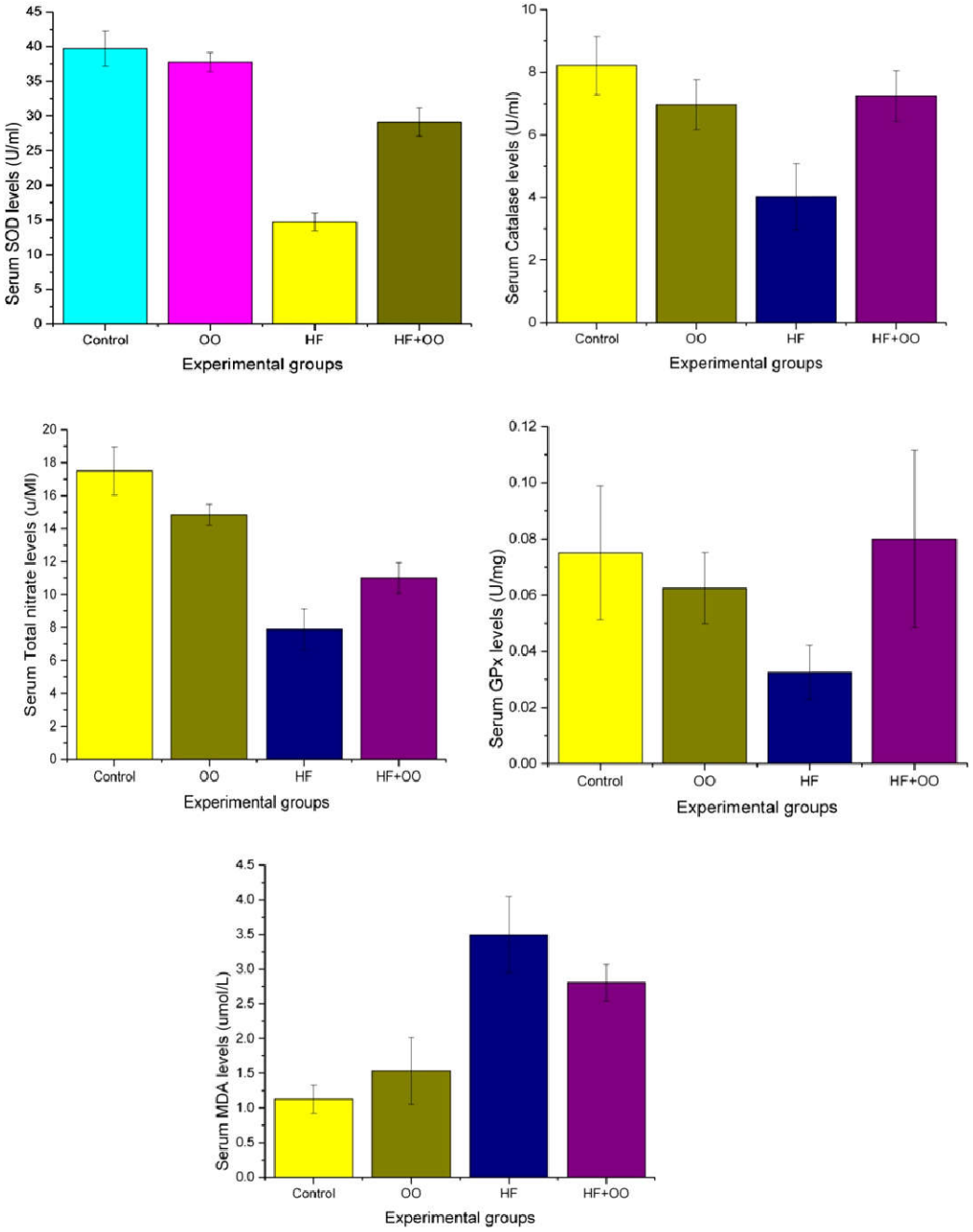

Figure 3a-e: Effect of Olive oil on serum antioxidant profile (SOD, CAT, MAD, GPx, Total nitrate) in diet induced obesity experimental model.

Table 3: **Effect of olive oil on Serum antioxidant enzyme levels in HFD induced obesity model**

Experimental groups	SOD(U/ml)	Catalase (U/ml)	GPx(U/mg)	MDA (umol/L)	Total nitrate (u/Ml)
Control	39.70±2.55	8.21±0.93	0.07±0.02	1.12±0.20	17.50±1.46
Olive oil	37.77±1.36	6.97±0.79	0.06±0.01	1.53±0.48	14.84±0.63
High fat	14.72±1.28	4.03±1.05	0.03±0.009	3.49±0.54	7.89±1.23
High fat + Olive oil	29.11±2.02	7.24±0.80	0.08±0.03	2.80±0.26	10.99±0.93

All values were expressed in Mean ±SD

REFERENCES

1. Hossain, P., Kawar, B. & El Nahas, M. (2007). Obesity and diabetes in the developing world--a growing challenge. N Engl J Med 356, 213-215.
2. Bluher, M. (2009). Adipose tissue dysfunction in obesity. Exp Clin Endocrinol Diabetes,117(6),241-250.
3. Galic, S., Oakhill, J. S., & Steinberg, G. R. (2010). Adipose tissue as an endocrine organ. Mol Cell Endocrinol, 316(2), 129-139.
4. Warolin, J.; Coenen, K.R.; Kantor, J.L.; Whitaker, L.E.; Wang, L.; Acra, S.A.; Roberts, L.J., 2nd; Buchowski, M.S. (2013). The relationship of oxidative stress, adiposity and metabolic risk factors in healthy Black and White American youth. Pediatr. Obes. 20-25,
5. Codoñer-Franch, P.; Tavárez-Alonso, S.; Murria-Estal, R.; Tortajada-Girbés, M.; Simó-Jordá, R.; Alonso-Iglesias, E. Elevated advanced oxidation protein products (AOPPs) indicate metabolic risk in severely obese children. Nutr. Metab. Cardiovasc. Dis. 2012, 22, 237–243.
6. Alhazza I.M., 2007. Antioxidant and Hypolipidemic Effects of Olive Oil in Normal and Diabetic Male Rats. Saudi Journal of Biological Sciences, 14 (1): 69-74.
7. Amin A, Hamza AA. Oxidative stress mediates drug-induced hepatotoxicity in rats a possible role of DNA fragmentation. Toxicol,2005; 208: 367-375.
8. B. Vessby,(2000). "Dietary fat and insulin action in humans," *British Journal of Nutrition*, vol. 83,supplement 1, pp. S91–S96.
9. Benavente-Garcia, O., J. Castillo, J. Lorente and M. Alcaraz, (2002). Radioprotective effects in vivo of phenolics extracted from Oleaeuropaea L. leaves against Xray-induced chromosomal damage: Comparative study versus several flavonoids and sulfur-containing compounds. J. Med. Food, 5: 125-135
10. Bouaziz, M. and S. Sayadi, (2005). Isolation and evaluation of antioxidants from leaves of a tunisian cultivar olive tree. Eur. J. Lipid Sci. Technol., 107: 497-504
11. Dandona, P.; Ghanim, H.; Chaudhuri, A.; Dhindsa, S.; Kim, S.S. (2010). Macronutrient intake induces oxidative and inflammatory stress: Potential relevance to atherosclerosis and insulin resistance. Exp. Mol. Med. 42, 245–253.
12. Falck-Ytter, Y.; Younossi, Z.M.; Marchesini, G.; McCullough, A.J. (2001). Clinical features and natural history of nonalcoholic steatosis syndromes. Semin. Liver Dis. 21, 17–26.
13. Gimeno E, Fitó M, Lamuela-Raventós RM, Castellote AI, Covas M, Farré M, de La Torre-Boronat MC, López-Sabater MC. (2002). Effect of ingestion of virgin olive oil on human low-density lipoprotein composition. Eur J ClinNutr. ;56(2):114-20
14. Gorinstein S, Leontowicz H, Lojek A, Leontowicz M, Ciz M, Krzeminski R, Gralak M, Czerwinski J, Jastrzebski Z, Trakhtenberg S, Grigelmo-Miguel N, Soliva-Fortuny R, Martin-Belloso O. J Agric Food Chem. (2002). 9;50(21):6102-8.
15. Haskins K, Bradley B, Powers K.(2003). Oxidative stress in type 1 diabetes. Ann N Y Acad Sci; 1005:43–54
16. Higuchi, M.; Dusting, G.J.; Peshavariya, H.; Jiang, F.; Hsiao, S.T.; Chan, E.C.; Liu, G.S. (2013). Differentiation of human adipose-derived stem cells into fat involves reactive oxygen species and forkhead box o1 mediated upregulation of antioxidant enzymes. Stem. Cells Dev. 22, 878–888.
17. Hu, F.B., (2003). The Mediterranean diet and mortality-olive oil and beyond. New Engl. J. Med., 348: 2595-2596.
18. I, J. Martins and T. G. Redgrave, "Obesity and post-prandial lipid metabolism. Feast or famine?"*Journal of Nutritional Biochemistry*, vol. 15, no. 3, pp. 130–141, 2004.
19. I.M Alhazza and Samir A. E Bashandy. (2007). Hypoglycemic , hypolipidemic, antioxidant and male sexual improvement potentials of olive oil in allozan treated rats. Journal of pharmacology and toxicology 2(5) : 427-436.
20. Jones P.J.H., Demonty I., Chan Y.M., Herzog Y. and Pelled D., (2007). Fish-oil esters of plant sterols differ from vegetable-oil sterol esters in triglycerides lowering, carotenoid bioavailability and impact on plasminogen activator inhibitor-1(PAI-1) concentrations in hypercholesterolemic subjects. Lipids in Health and Disease, 6: 28-37.
21. Keys A, Menotti A, Karvonen MJ, et al. (1986). The diet and 15-year death rate in the Seven Countries Study. *Am J Epidemiol* ;124:903-915.
22. Khayyal, M.T., M.A. El-Ghazaly, D.M. Abdallah, N.N. Nassar, S.N. Okpanyi and M.H. Kreuter, (2002). Blood pressure lowering effect of an olive leaf extract (Oleo europaea) in L-NAME induced hypertension in rates. Arzneimittel-Forschung/Drug Res., 52: 797-802.
23. Klop, J. W. F. Elte, and M. C. Cabezas, (2013). "Dyslipidemia in obesity: mechanisms and potential targets," *Nutrients*, vol. 5, no. 4, pp. 1218–1240.

24. Komaki, F., E. Yamaguchi, S. Maru, M. Kinoshita, K. Kakehi, Y. Ohta and Y. Tsukada, (2003). Identification of anti-alpha-amylase components from olive leaf extracts. Food Sci. Technol. Res., 9: 35-39
25. Lee, H.; Lee, Y.J.; Choi, H.; Ko, E.H.; Kim, J.W. (2009). Reactive oxygen species facilitate adipocyte differentiation by accelerating mitotic clonal expansion. J. Biol. Chem. 284, 10601–10609.
26. Lopez-Miranda, J., F. Perez-Jimenez, E. Ros, R. De Caterina, et al., (2010). "Olive oil and health: summary of the II international conference on olive oil and health consensus report, Jaen and Cordoba (Spain) 2008." NutrMetabCardiovasc Dis 20 (4): 284-294.
27. Lorke, D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983; 54, 275–287.
28. Louka, M.L., Habib, H.Z., Youssef, M.H.M. and Nassef, N.A.H. (2012) Effect of Olive Oil Supplementation on PAI-1 Expression in Old Rats. The Journal of American Science, 8, 317-321
29. M. Mbikay, "Therapeutic potential ofMoringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review," *Frontiers in Pharmacology*, vol. 3, no. 24, pp. 1–12, 2012.
30. Mukundh NB, Muralidharan P, Balamurugan G: Antihyperlipidemic activity of Pedalium murex (Linn) fruits on high fat diet fed rats. Int J Pharmacol 2008, 4:310–313.
31. P. Haley, M. M. Gonzales, T. Tarumi, and H. Tanaka, "Dyslipidemia links obesity to early cerebral neurochemical alterations," *Obesity*, vol. 21, no. 10, pp. 2007–2013, 2013.
32. Paoli A., Cenci L., Grimaldi K..A., (2011). Effect of ketogenic Mediterranean diet with phyto extracts and low carbohydrates/high protein meals on weight, cardiovascular risk factors, body composition and diet compliance in Italian council employees. Nutr J., 10:112- 120.
33. Pérez-Martínez P., García-Ríos A., Delgado-Lista J., Pérez-Jiménez F., López-Miranda J.,(2011). Mediterranean diet rich in olive oil and obesity, metabolic syndrome and diabetes mellitus. Curr Pharm., 17(8): 769-77.
34. Poudyal, H., F. Campbell and L. Brown, (2010). Olive leaf extract attenuates cardiac, hepatic and metabolic changes in high carbohydrate-, high fat-fed rats. J. Nutr., 140: 946-953.
35. Raja S., Ahmed K., Kumar V., Mukherjee K., Bandyopadhyay A., Mukherjee P., (2007). Antioxidant effect of Cytisus scorparius against carbon tetrachloride treated liver injury in rat. J. Ethnopharmacol., 2007, 109, 41–47
36. Rosa Martha Perez Gutierrez, Diana Madrigales Ahuatzi, Maria del Carmen Horcacitas, Efren Garcia Baez, Teresa Cruz Victoria, and Jose Maria Mota-Flores,(2014). "Ameliorative Effect of Hexane Extract of Phalaris canariensis on High Fat Diet-Induced Obese and Streptozotocin-Induced Diabetic Mice," Evidence-Based Complementary and Alternative Medicine, vol. 20. 35-40
37. Serra, D.; Mera, P.; Malandrino, M.I.; Mir, J.F.; Herrero, L. (2012). Mitochondrial fatty acid oxidation in obesity. Antioxid. Redox Signal. doi:10.1089/ars.2012.4875.
38. Simon-Giavaritti KA, Giavarotti L, Gomes LF, Lima AF, Veridin A M,Garcia EA, Mora OA, Fernandez V, Videla LA, Junqueira VB. (2002). Enhancement of lindance-induced liver oxidative stress and hepatotoxicity by thyroid hormone is reduced by gadolinum chloride. FreeRad Res, 36:1033-1039.
39. Somova, L.I., F.O. Shode and M. Mipando, (2004). Cardiotonic and antidysrhythmic effects of oleanolic and ursolic acids, methyl maslinate and uvaol. Phytomedicine, 11: 121-129.
40. Wanless IR, Lentz JS: (1990). Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology, 12:1106–1110