Original Article

EFFECT OF DIETARY SUBSTITUTION OF HEATED AND UN-HEATED EXTRA VIRGIN OLIVE OIL ON OXIDATIVE STRESS IN STREPTOZOTOCIN-DIABETIC RATS

Muhammad Javad Yousaf, Abdul Khaliq Naveed, Tausif Ahmed, Shakir Khan, Zahid Azeem

Army Medical College Rawalpindi

ABSTRACT

Objectives: To evaluate and compare the effects of un-heated extra virgin olive oil and heated extra virgin olive oil on oxidative stress in diabetic rats.

Study Design: Randomized Control Trial

Place and Duration of Study: Department of Biochemistry, Army Medical College, Rawalpindi in collaboration with Centre for Research in Experimental & Applied Medicine, Army Medical College, Rawalpindi and National Institute of Health, Islamabad from March 2010 to June 2011.

Material and Methods: One hundred and twenty albino rats of Sprague-Dawley strain weighing 200-250 g were randomly divided into three groups of 40 rats each. Rats were made diabetic by injecting streptozotocin. Group 1, group 2 and group 3 were given normal rodent diet, un- heated extra virgin olive and heated extra virgin olive oil supplemented diet respectively for 6 weeks. At the end of experimentation, plasma malondialdehyde level (biomarker of oxidative stress) was measured.

Results: Serum malondialdehyde level was significantly lower in group-2 as compared to group-1 ($p < 0.05$) and group-3 ($p < 0.05$), where as the difference in serum malondialdehyde levels of group-1 and group-3 was insignificant ($p > 0.05$).

Conclusion: Un-heated-extra virgin olive oil can significantly improve oxidative stress in diabetic rats.

Keywords: Diabetes mellitus, Extra virgin olive oil, Oxidative Stress, Sprague-Dawley rats

INTRODUCTION

Diabetes mellitus (DM) is a continuous source of oxidative stress to the body. The uncontrolled hyperglycemia acts as a major player of auto-oxidation of glucose, glycosylation of proteins and lipids leading to production of advanced glycation end products. All these biochemical alterations consequently result in production of free radicals that subsequently increase the oxidative stress, responsible of all the complications owed to DM. The consequences of glycoxidative stress (GOS) include; damage to DNA, lipids, proteins, disruption in cellular homeostasis and accumulation of damaged molecules. These damaged molecules disrupt endothelial cells and cardiomyocytes and impair cardiovascular reactivity.

In diabetes, inculcation of benign therapeutics with minimal side effects is the need of the day. Extra virgin olive oil (EVOO) is the most superior quality of unrefined edible olive oil, containing high concentration of polyphenolic antioxidants. It serves as source of at least 30 phenolic compounds that include hydroxytyrosol, tyrosol and oleuropein, having good antioxidant activity both in-vivo and in-vitro[1]. Olive oil is an integral component of Mediterranean diet and can lower risk of ischemic heart disease, cancer and has anti-inflammatory action which can positively impact illnesses like arthritis, hypertension, diabetes and asthma.

EVOO is used in different food preparation and impart delicious flavor and texture to food. EVOO is mostly consumed raw in salads, toasts and other foodstuffs, but often may also be used after domestic heating, such as pan frying, baking, cooking, grilling and microwave heating, which exposes it to excessive heat. Heating of oil results in thermal and oxidative decomposition with the formation lipid oxidation products, anti

Correspondence: Maj Gen Abdul Khaliq Naveed, Dean and Head of Biochemistry Department, Army Medical College Rawalpindi
Email:khaliqnaveed2001@yahoo.com
Received: 09 April 2012; Accepted: 02 May 2012

nutritional factors, volatile and non-volatile decomposition products, desirable or undesirable flavor compounds, mutagens and carcinogens[2,3]. Heating also damages essential amino acids, fatty acids and naturally occurring antioxidants which will change the flavor stability and quality of the oil[3,4]. This damage varies depending on extent, duration and mode of heating.

In view of the potential hazardous effect of heated oil on health, this study was planned in rats to determine the effects of un-heated and heated EVOO on oxidative stress in diabetes. These diabetic rats were supplemented with normal rodent diet, un-heated EVOO and heated-EVOO rich diet for 6 weeks before getting final notion. Almost all of olive oil studies in diabetes are of Western origin and are on un-heated EVOO. This is exclusively a new study design which has not been performed in our set up so far.

MATERIALS AND METHODS

This randomized control trial was performed in the Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi in collaboration with Centre for Research in Experimental & Applied Medicine (CREAM), Army Medical College and National Institute of Health (NIH), Islamabad. This study was carried on one hundred and twenty, 60-90 days old Albino rats of Sprague Dawley strain, weighing between 200-250g. Rats were obtained from the animal house of NIH, Islamabad and were kept at its animal house. Total duration of study was 6 weeks. Rats were made diabetic by injecting streptozotocin (STZ) 40-mg/kg/body weight intraperitoneally dissolved in citrate buffer. On the third day, their fasting blood glucose was measured by taking blood from tail veins under aseptic measures. Rats with blood glucose level more than 126 mg/dl were considered as diabetic and were used in study. The diabetic rats were randomly divided into three groups of 40 rats each using random number table:

Group-1 (Diabetic Control Group): Forty diabetic rats in group–I served as control animals for the experimental groups of study. They were fed standard pelleted diet as per requirement, prepared at NIH, Islamabad, according to the international standards for 06 weeks.

Group- 2 (Diabetic Experimental Group): Forty diabetic rats in group–2 fed with un-heated-EVOO supplemented diet as per requirement, prepared at NIH Islamabad for 06 weeks. Un-heated EVOO supplemented diet was the diet containing 100 grams of un-heated EVOO per kilogram of pelleted diet.

Group- 3 (Diabetic Experimental Group): Forty diabetic rats in group-2 fed with heated-EVOO supplemented diet as per requirement, prepared at NIH Islamabad for 06 weeks. EVOO was heated at smoke point for 15 minutes. Heated EVOO supplemented diet was the diet containing 100 grams of this highly heated EVOO per kilogram of pelleted diet.

Rats were kept under standard conditions with a daily photo period of 12 hours light and 12 hours dark at 23 ± 2°C. Five animals were kept in one iron cage. All groups had free access to food and water. At the end of experiment, fasting blood samples were drawn through intracardiac puncture for measurement of plasma malondialdehyde (MDA). MDA (biomarker of oxidative stress), was measured by Enzyme-linked immunosorbent assay (ELISA) by using TBARS assay kit.

Statistical Analysis

The data was entered and analyzed using SPSS version 15.0. The arithmetic mean and standard deviation (SD) of MDA was calculated. The statistical significance of difference across the groups was determined by applying one way analysis of variance (ANOVA) followed by Post Hoc Tukey HSD test. The difference was considered significant if p value was found less than 0.05.

RESULTS

Plasma MDA levels of group-1, group-2 and group-3 rats were significantly different ($p<0.05$) (figure-1). MDA level was significantly lower in group-2 as compared to group-1 ($p< 0.05$) and group-3 ($p< 0.05$), whereas the difference in MDA levels of group-1 and group-3 was insignificant ($p>0.05$) (Table-1).

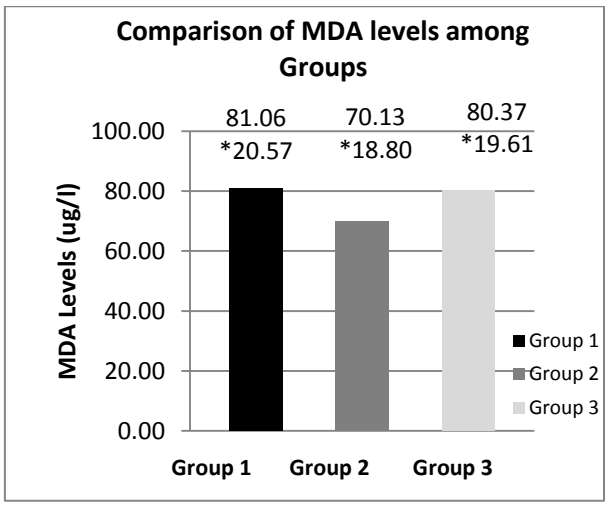

Figure-1: Comparison of plasma MDA among group-1, group-2 and group-3.
*SD

Table-1: Comparison of groups (ANOVA followed by Tukey HSD)

Paired on Post-Hock Comparison	Mean Difference	*p*- value
Group 1 vs Group 2	10.93	0.034 *
Group 1 vs Group 3	0.69	0.979 NS
Group 2 vs Group 3	10.24	0.041 *

* Significant Difference ($p < 0.05$)
NS Insignificant Difference ($p > 0.05$)

DISCUSSION

In DM there is increased generation of reactive oxygen species (ROS). ROS have a very short half-life and cannot remain as such and react rapidly with DNA, protein, and lipids, thereby leading to oxidative damage. Anti-oxidants can cancel out the damaging effects of the oxidants by decreasing their production or increasing their excretion. It has been reported that each 100 g of EVOO contains about 50 mg of polyphenols. MDA is the marker of oxidative stress which was significantly decreased in group-2 but no significant change in group-3. Thus un-heated EVOO had strong antioxidative action but heated EVOO had poor antioxidant action.

These results are in synchronization with Goya *et al*. and Nakbi *et al*. research studies in human and rats, which have proven antioxidative action of olive oil[5,6]. Our study correlates with multiple series of research work carried by Owen *et al*. and De la Puerta *et al*. in which they found that phenolic components scavenge ROS under natural and chemically simulated oxidative stress conditions[7,8]. Salvini *et al.* and Bogani *et al.* concluded from their studies in human that total plasma antioxidant activity had increased after the ingestion of olive oil phenolic compounds, which is in accordance to our results[9,10]. In conjugation to these findings, Loru *et al.* and Paiva-Martins *et al.* from their experimental studies showed that phenolic compounds of olive oil reduce oxidative damage to red blood cells and renal cells[11,12].

Heated EVOO had poor antioxidant activity, which postulates the view that excessive heating has caused oil degradation and damage to it its polyphenols component. These thermal biophysical changes occurring due to hydrolysis, oxidation and polymerization impart organoleptic quality of EVOO. These findings are in synchronization with the studies of Andrikopoulos *et al.*, Allouche *et al.* and Bendini *et al.* which showed that excessive heating of olive oil caused damage to phenolic compounds[13,14,15]. Our study also correlates with those conducted by Brenes *et al.* in which they applied different method of heating to olive oil like frying, microwave heating and boiling with water in a pressure cooker, all caused damage to phenolic components[4].

Our study correlates with research work carried by Gómez-Alonso *et al.* in which they found that upon deep frying anti-oxidative capacity of olive oil is reduced[16]. These finding are in synchronization with the studies of Kamsiah *et al*. who found that heated vegetables oil had no effect on oxidative stress[17]. Our study is also in accordance with Adam *et al.* in post-menopausal rat, which showed that ingestion of multiple heated palm oil even increased oxidative stress than normal[18].

In contrary, a randomized cross-over study was carried by Moschandrea *et al.* in Greek smokers, which showed that phenolic components of olive oil had no effect on markers of oxidative stress[19]. Findings in this study also contradicts with Vissers *et al.* randomized cross-over intervention studies in

healthy persons, which showed that phenolic components of olive oil did not affect LDL or HDL oxidation and other markers of oxidative stress[20]. Findings in the present study are also partially in contrast with Casal *et al.* heat stability experiments of olive oil. The results showed that mostly olive oil is clearly resistant to deep frying conditions[21].

CONCLUSION

Present study substantiates the observation that un-heated-extra virgin olive oil has antioxidative effect in diabetics. Hence, it can easily be contemplated that EVOO in its un-heated form is more beneficial than in heated form in diabetics.

REFERENCES

1. Tripoli E, Giammanco M, Tabacchi G, Di Majo D, Giammanco S. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. Nutr Res Rev. 2005; 18(1):98-112.
2. Purushothama S, Ramachandran HD, Narasimhamurthy K, RainaPL. Long-term feeding effects of heated and fried oils on hepatic antioxidant enzymes, absorption and excretion of fat in rats. Mol Cell Biochem. 2003; 247 (1-2): 95-9.
3. Billek G. Heated oils – chemistry and nutritional aspects. Nutr Metab. 1980;24 Suppl 1:200-10.
4. Brenes M, García A, Dobarganes MC, Velasco J, Romero C. Influence of thermal treatments simulating cooking processes on the polyphenol content in virgin olive oil. J Agric Food Chem. 2002 Oct 9;50(21):5962-7.
5. Goya L, Mateos R, Bravo L. Effect of the olive oil phenol hydroxytyrosol on human hepatoma HepG2 cells: Protection against oxidative stress induced by tert-butylhydroperoxide. Eur J Nutr. 2007; 46 (2): 70-8.
6. Nakbi A, Tayeb W, Grissa A, Issaoui M, Dabbou S, Chargui I, Ellouz M, et al. Effects of olive oil and its fractions on oxidative stress and the liver's fatty acid composition in 2, 4-Dichlorophenoxyacetic acid-treated rats. NutrMetab (Lond). 2010 Oct 29;7:80.
7. De la Puerta R, Martinez Dominguez ME, Ruiz-Gutierrez V, Flavill JA, Hoult JR. Effects of virgin olive oil phenolics on scavenging of reactive nitrogen species and upon nitrergic neurotransmission. Life Sci. 2001;69:1213–22.
8. Owen RW, Giacosa A, Hull WE, Haubner R, Spiegelhalder B, Bartsch H. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. Eur. J. Cancer. 2000;36:1235–47.
9. Bogani P, Galli C, Villa M, Visioli F. Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. Atherosclerosis. 2007;190:181–6.
10. Salvini S, Sera F, Caruso D, Giovannelli L, Visioli F, et al. Daily consumption of a high-phenol extra-virgin olive oil reduces oxidative DNA damage in postmenopausal women. Br. J. Nutr. 2006;95:742–51.
11. Paiva-Martins F, Fernandes J, Rocha S, Nascimento H, Vitorino R, Amado F, Borges F, Belo L, Santos-Silva A. Effects of olive oil polyphenols on erythrocyte oxidative damage. Mol. Nutr. Food Res. 2009;53:609–16.
12. Loru D, Incani A, Deiaa M, Corona G, Atzeri A, Melis MP, Rosa A, Dessi MA. Protective effect of hydroxytyrosol and tyrosol against oxidative stress in kidney cells. Toxicol. Ind. Health. 2009;25:301–10.
13. Andrikopoulos NK, Dedoussis GV, Falirea A, Kalogeropoulos N, Hatzinikola HS. Deterioration of natural antioxidant species of vegetable edible oils during the domestic deep-frying and pan-frying of potatoes. Int J Food Sci Nutr. 2002; 53(4):351-63.
14. Bendini A, Valli E, Cerretani L, Chiavaro E, Lercker G. Study on the effects of heating of virgin olive oil blended with mildly deodorized olive oil: focus on the hydrolytic and oxidative state. J Agric Food Chem. 2009; 57(21):10055-62.
15. Allouche Y, Jiménez A, Gaforio JJ, Uceda M, Beltrán G. How heating affects extra virgin olive oil quality statuses and chemical composition. J Agric Food Chem. 2007: 14;55(23): 9646-54
16. Gómez-Alonso S, Fregapane G, Salvador MD, Gordon MH. Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. J Agric Food Chem. 2003: 29;51(3):667-72.
17. Kamsiah J , M. Norhayati, G. Norzana, U. Nor Aini, S. Ima- Nirwana. Effects of heated vegetable oils on serum lipids and aorta of ovariectomized rats. Pak. J. Nutr. 2006; 5 (1): 19-29.
18. Adam SK, Soelaiman IN, Umar NA, Mokhtar N, Mohamed N. Effects of repeatedly heated palm oil on serum lipid profile, lipid peroxidation and homocysteine levels in a post-menopausal rat model. Mcgill J Med. 2008; 11(2):145-51.
19. Moschandreas J, Vissers MN, Wiseman S, van Putte KP, Kafatos A. Extra virgin olive oil phenols and markers of oxidation in Greek smokers: randomized cross-over study. Eur J Clin Nutr. 2002; 56 (10): 1024-9.
20. Vissers MN, Zock PL, Wiseman SA, Meyboom S, Katan MB. Effect of phenol-rich extra virgin olive oil on markers of oxidation in healthy volunteers. Eur. J. Clin. Nutr. 2001; 55: 334–41
21. Casal S, Malheiro R, Sendas A, Oliveira BP, Pereira JA. Olive oil stability under deep-frying conditions. Food Chem Toxicol. 2010; 48(10):2972-9