ORIGINAL ARTICLE

Antioxidant Effect of Extra Virgin Olive Oil and Apple Cider Vinegar in Type 2 Diabetic Rat Model

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ABSTRACT

Objective: Comparison between the antioxidant effects of extra virgin olive oil and apple cider vinegar in rats induced with diabetes mellitus type 2.

Study Design: Randomized controlled trial.

Place and Duration of Study: This Study was conducted in Postgraduate Medical Institute, Animal House Lahore, Pakistan from May 2021 to June 2021.

Methods: In this study, 40 male Sprague Dawley rats were divided into 4 groups i.e., Group I was NC (negative control), Group II PC (positive control), Group III EVOO (Extra virgin olive oil) and Group IV (Apple cider vinegar), each group having 10 rats. Diabetes was induced in all rats except the rats of NC group at the start of the study by intraperitoneal administration of injection nicotinamide, followed by injection Streptozosin (STZ) after 15 minutes. Group III was given 1ml/100gBW/ day EVOO and Group IV was given 2ml/kg BW/day diluted ACV with distilled water in 1:5 orally for 4 weeks. Sampling was done after 4 weeks for determination of oxidative stress markers Malonaldehyde, Superoxide dismutase and Total antioxidant status in serum.

Results: Intake of EVOO and ACV showed reduction in serum Malonaldehyde levels as compared to positive control group with *P*-values 0.000 and 0.014 respectively. Serum superoxide dismutase activity and Total antioxidant status were lowest in the positive control group while both the treatment groups showed significant enhancement in these parameters as compared to positive control group with *P*-value = 0.000.

Conclusion: Both extra virgin olive oil and apple cider vinegar have antioxidant effects in type 2 diabetic rats. However, Extra virgin olive oil is more potent.

Keywords*: Antioxidant Effects, Diabetes Mellitus, Olive Oil.*

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Introduction

Type 2 diabetes mellitus is differentiated by resistance to insulin at the insulin receptors and changed metabolism and dyslipidemia.[1] Diabetes mellitus has surfaced as an issue since past few eras, leading to colossal drain on health resources.[2] There were estimated 451 million people diabetics around the world and 693 million to have it by the 2045.[3] An estimated 26.3% adults are suffering from this condition in Pakistan.[4]

In diabetes, persistent high blood sugar levels leading to the generation of ROS. The imbalance between free radicals and antioxidants in individuals with diabetes contributes to the harm of various organs.[5] Free radicals cause alterations in structure and functions of cellular components i.e., cell

membrane and biomolecules such as lipids, nucleic acid and proteins. Malondialdehyde (MDA) is produced by lipid peroxidation of membrane lipids and is a prime marker of cell injury. Lipid peroxidation is involved in development of micro and macro vascular complications of diabetes. Oxidative damage is prevented by enzymes such as catalase (CAT), glutathione peroxidase and superoxide dismutase (SOD) and non-enzymatic components of total antioxidant status (TAS) for example ascorbic acid and tocopherol but there is a significant decline TAS in DM.[6]

Diabetic patients are currently being treated by insulin and oral hypoglycemic agents. The challenges associated with insulin use and the side effects of medications are driving an increased interest in natural treatment options for diabetes.[7] Among these are phenolic compounds, which are recognized for their hypoglycemic and antioxidant properties. Extra virgin olive oil is particularly high in phenolic compounds compared to other types of olive oil.[8] Apple cider vinegar, which is made through a two-step fermentation process, contains 5-6% acetic acid.[9] The phenolic compounds in ACV inhibit generation of ROS antioxidant and lower blood glucose levels.[10] Additionally, apple cider vinegar exhibits hepatoprotective properties due to phloretin, which plays a crucial role in non-enzymatic protection against oxidative stress-induced liver damage.[11]

This study is aimed to compare the antioxidant effect of EVOO and ACV in diabetic rats by determining serum levels of oxidative stress markers.

Methods

This experimental study, with a duration of 40 days, was conducted at the Animal House of Postgraduate Medical Institute, Lahore from May 2021 to June 2021. Approval was taken by Ethical Committee of the institute on dated: 14th March 2021 vide letter no: 00/20/5/2019.

Sample size was calculated by using formula;

$$n = \frac{\sigma^2(z_{1-\alpha/2} + z_{1-\beta})^2}{(\mu_o - \mu a)^2}$$

The level of significance (α) = 5% and level of confidence = 95%. Values of μ_o & μ_a were obtained from ALT levels from the result of Balamash KS et al. Sample size calculated was 10 in each group.[12]

40 adult healthy male rats of (180-200 gram) selected by simple random sampling were bought from local market in Lahore. Rats with any ailment signs were excluded from the study.

Before starting the experiments, the rats were allowed a week to acclimate in the animal house. They were in appropriately sized, cages at a room temperature of 22-24^{0}C and provided with rat chow and water orally. Following this acclimatization period, the rats were randomly assigned into four groups (10 rats per group).

Type 2 diabetes mellitus was induced in all rats except those in the normal control by administering intraperitoneal injections of nicotinamide using a 26-gauge needle, followed by an injection of streptozotocin (STZ). Rats with blood glucose levels exceeding 200 mg/dL, as measured by a handheld glucometer, 72 hours after the STZ injection, were considered diabetic.[13]

Both control groups, Group I (NC) and Group II (PC), received 1 ml/100 g body weight/day of distilled water orally. Group III (EVOO) was administered 1 ml/100 g body weight/day of extra virgin olive oil (Spain), while Group IV (ACV) was given 2 ml/kg body weight/day of diluted apple cider vinegar mixed with distilled water.[12-14]

At the end of 4th week cardia puncture was performed to collect 4 ml blood of each rat after overnight fast. Light anesthesia with chloroform was used to collect samples by using 26-gauge needle. Sample tubes were placed in straight standing position for 30 minutes at room temperature and then centrifuged. Serum MDA levels were measured by ELISA while serum SOD & TAS were measured by colorimetric method.

Results

Very highly significant difference in serum stress markers (MDA, SOD and TAS) levels was noted when comparison among groups was done by Kruskal Wallis *H* test (*P*=0.000) (Table-1) because data was non normally distributed. Serum MDA levels of NC rats were significantly less than the PC rats with a *P* = 0.000. Serum MDA levels of ACV and EVOO groups were significantly less than PC group with *P*-values of 0.000 and 0.014 respectively. (Table-2).

Serum SOD levels of NC rats were higher than the PC rats with *P* = 0.000. Serum TAS of PC rats were very

highly significantly lower than the NC rats with a *P* = 0.000. Comparison of serum Total Antioxidant Status of both the groups with Positive Control group had *P*-value of 0.0000. (Table- 2).

Table-1: Comparison of serum stress markers (malondialdehyde, superoxide dismutase and total antioxidant status) levels

Groups		Malondialdehyde (nmol/mL)	Superoxide dismutase (U/mL)	Total antioxidant status (U/mL)
	Median	1.35	22.80	4.25
	(IQR)	(1.20-1.52)	(22.80-23.62	(3.92-4.47)
	Median	4.30	11.70	1.24
	(IQR)	(3.87-4.82)	(11.20-12.65)	(0.49-2.10)
	Median	2.50	17.20	4.68
	(IQR)	(2.20-2.57)	(16.67-17.77)	(4.53-6.41)
	Median	3.50	16.60	3.82
	(IQR)	(3.27-3.92)	(16.15-17.05)	(2.99-4.07)
P-value		0.000***	0.000***	0.000***

****P* value very highly significant. PC: Positive control NC: Normal control, EVOO: Extra virgin Olive oil, ACV: Apple Cider vinegar

Table-2: Multiple comparison of serum stress markers (malondialdehyde, superoxide dismutase and total antioxidant status) levels among groups

Groups		Malondialdehyde (nmol/mL)	Superoxide dismutase (U/mL)	Total antioxidant status (U/mL)
Group I (NC)	Median	1.35	22.80	4.25
	(IQR)	(1.20-1.52)	(22.80-23.62)	(3.92-4.47)
		[2]p=0.000***	[2]p=0.000***	[2]p=0.000***
Group II (PC)	Median	4.30	11.70	1.24
	(IQR)	(3.87-4.82)	(11.20-12.65)	(0.49-2.10)
Group III (EVOO)	Median	2.50	17.20	4.68
	(IQR)	(2.20-2.57)	(16.67-17.77)	(4.53-6.41)
		[1]p=0.000***	[1]p=0.000***	[1]p=0.006**
		[2]p=0.000***	[2]p=0.000***	[2]p=0.000***
Group IV (ACV)	Median	3.50	16.60	3.82
	(IQR)	(3.27-3.92)	(16.15-17.05)	(2.99-4.07)
		[1]p=0.000***	[1]p=0.000***	[1]p=0.028*
		[2]p=0.014*	[2]p=0.000***	[2]p=0.000***

**P*-value significant. PC: Positive control NC: Normal control, EVOO: Extra virgin Olive oil, ACV: Apple Cider vinegar

Discussion

The Oxidative stress has a crucial part in pathogenesis of T2DM and its complications. This study was conducted to study the antioxidant effect of ACV and EVOO in rats induced with diabetes. There are many markers of oxidative stress including ROS themselves. Since ROS have short half-lives, it is appropriate to determine oxidative stress by measuring the products of oxidation reactions. Free radicals react with lipid bilayer of plasma membrane resulting in lipid peroxidation and MDA is produced as a result which is more stable than ROS.[15,16]

In our study, DC group showed the highest levels of serum MDA among all the groups. Both the groups that were given treatment i.e., EVOO and ACV showed reduction in serum MDA levels as compared to DC group with p values of 0.000 and 0.014 respectively. Serum SOD activity and serum TAS were lowest in the DC group among all the groups. While both the groups that were treated had very highly significant enhancement in serum SOD activity as well as TAS (*P* values < 0.001) as compared to DC group.

Significantly raised MDA levels were noted in T2DM

with and without complications in previous studies, confirming results of our study.[15,16] Diabetes lowered the activities of antioxidant enzymes, such as activity of SOD in the study of Karabag-Coban F et al. It was suggested that reduction in activity of enzymes is due to their enhanced utilization in combating oxidative stress in diabetic group. Another study is in accordance with our study, reporting decrease in plasma SOD activity as well as TAS in type 2 diabetics as compared to normal participants.[17]

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Olive oil contains abundant bio phenols like HT, oleuropein and their derivatives which have antioxidative effect.[19] Olive oil helps to increase serum total antioxidant capacity by enhancing the level and activity of antioxidant enzymes. Olive oil consumption leads to increase in MUFA content in LDL, MUFA are more resistant to oxidation than polyunsaturated fatty acids, preserving antioxidant compounds bound to LDL.[20] Temiz and Temur reported that polyphenolic compounds present in olive leaf extract increased the activity of antioxidant enzymes like SOD, CAT and glutathione activity in the diabetic rats of their study. It was suggested that these polyphenols also increase the expression of enzymes SOD and CAT at the level of transcription.[21] Karabag-Coban F et al. indicated decreased lipid peroxidation resulting in decreased MDA levels in oleuropein treated diabetic animals. They attributed this achievement to the ability of oleuropein (an important component of olive oil) to decompose and trap free radicals before reaching the cellular targets. Moreover, oleuropein was found to reestablish SOD activity in the treatment group of their study.[17] However, Atefi M et al. noted non-significant effect of olive oil on MDA level in type 2 diabetics; they have attributed their results to short duration of their study.[22]

The antioxidant effects of ACV shown in our study are in accordance to the properties of ACV reported by previous studies. Polyphenols present in vinegars provide antioxidant properties to vinegars because they have capacity to remove free radicals, reduce oxidants and chelate transition metal ions. The benzene ring of polyphenols blocks free radical chains and the hydroxyl group on benzene ring chelate transition metal ions, preventing oxidation reaction. Moreover, phenolic hydroxyl group can be oxidized to quinone, thus polyphenols also have a reducing effect.[23] Gheflati A et al. noted in their study that consumption of apple vinegar by type 2 diabetics had inverse relation with MDA level and they attributed this finding to the ability of flavonoids to scavenge ROS. Moreover, total antioxidant capacity of the vinegar group was also assessed in their study which was enhanced as compared to diabetic control group, confirming our results.[24] Abdulrauf RA et al. observed and reported in their study that decline in lipid peroxidation as evidenced by fall in serum MDA level by treating oxidative stress by ACV and they suggested catechins present in ACV to be responsible for reduction in LDL oxidation. Their treatment group also showed an increase in serum SOD activity, attributed to up regulation of the release of endogenous SOD by ACV as well as to the enhancement of SOD synthesis by the minerals (potassium, zinc and copper) present in ACV. Moreover, it was also suggested that phenolic acids and vitamins in ACV can remove superoxide anion, resulting in potent antioxidant activity.[25]

Conclusion

This study concludes that apple cider vinegar as well as extra virgin olive oil have antioxidant effect in rat model of type because both lowered the oxidative stress marker and increasing the antioxidants. The effect of EVOO was more definite.

Limitations: Large clinical trials are needed to confirm these findings in human settings.

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Conflict of Interest: The authors declare no conflict of interest.

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Authors Contribution

RN: Idea conception, study designing, data collection, data analysis, results and interpretation
MS: Idea conception, study designing, data collection, data analysis, results and interpretation
HNL: Idea conception, study designing, data collection, manuscript writing and proofreading
GM: Idea conception, study designing, data collection
SN: Idea conception, study designing, data collection
ZRC: Data analysis, results and interpretation, manuscript writing and proofreading