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

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ABSTRACT

Introduction: Type-2 diabetes mellitus, a major public health problem, is caused by insulin resistance. In diabetics, chronic hyperglycemia results in formation of reactive oxygen species. Oxidative stress in diabetic patients is responsible for hepatic damage because it causes activation of several pro-inflammatory mediators in liver. Hepatic complications of diabetes include fatty liver disease, necrosis, cirrhosis, carcinoma and liver failure.

Aims & Objectives: To determine and compare the hepatoprotective effect of extra virgin olive oil and apple cider vinegar in streptozotocin induced Type-2 diabetic rat model.

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

Material & 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 STZ after 15 minutes. Group III was given 1ml/100gBW/ day EVOO and Group IV was given 2ml/kgBW/day diluted ACV with distilled water in 1:5 orally for 4 weeks. Terminal sampling was performed at the end of the 4th week for estimation of liver enzymes (ALT, ALP and AST) in serum. Data were entered and analyzed in SPSS version 26. p value of ≤ 0.05 was considered significant.

Results: On comparison with PC group, both the treatment groups showed significant decrease in serum ALT levels with p values 0.026 for EVOO and < 0.001 for ACV. Serum ALP levels were also decreased significantly in both the treated groups with p value < 0.001 for EVOO as well as for the ACV group. However, reduction in serum AST levels was nonsignificant with p values 0.082 and 0.058 in EVOO and ACV groups respectively.

Conclusion: Both EVOO and ACV have hepatoprotective effect in Type-2 diabetic rats. However, ACV is more effective.

Keywords: Apple cider vinegar, Extra virgin olive oil, Type-2 diabetes

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder which is characterized by hyperglycemia, caused by impaired insulin secretion or insulin resistance. Type-2 diabetes mellitus is characterized by insulin insensitivity of the insulin receptors and involves changes in carbohydrate metabolism and dyslipidemia[1]. Type-2 diabetes mellitus has come out as a global epidemic for the past few decades, posing a huge health burden[2]. According to one estimation there were 451 million people with diabetes worldwide in 2017 and 693 million people are expected to have this disorder by the year 2045[3]. About 26.3% of the adult local population is diabetic in Pakistan[4]. In diabetes, long term hyperglycemia induces glucose auto-oxidation and non-enzymatic glycosylation, promoting production of reactive oxygen species. Imbalance of free radicals and antioxidants in diabetics is responsible for damage of various organs[5]. Oxidative stress promotes the activation of several pro-inflammatory mediators and production of glucose by hepatocytes in an abnormal way[6]. Hepatic enzymes leak out of the damaged hepatocytes, enter into blood stream, leading to fluctuations in the serum alkaline phosphatase (ALP), aspartate amino-transferase (AST) and alanine amino-transferase (ALT) levels. Fatty change in liver, necrosis, cirrhosis, hepatocellular carcinoma and hepatic failure may

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also occur[7]. Although, oral hypoglycemic agents are most commonly used drugs for the management of diabetes, but they have some adverse effects such as hypoglycemia, gastrointestinal disturbances, weight gain, lactic acidosis and retention of fluid[8]. Inconvenience in use of insulin and side effects of oral hypoglycemic agents are leading to an increase in usage of natural remedies for diabetes[9]. These remedies include phenolic compounds because they are known to have hypoglycemic and antioxidant effects. There are many plant derived foods which have phenolic compounds for example extra virgin olive oil (EVOO)[10] and apple cider vinegar (ACV)[11]. Olive oil is obtained by mechanical extraction of the olive fruits and has abundant polyphenols. Composition of olive oil depends on harvest conditions, processing and preservation methods. Extra virgin olive oil is the richest in phenolic compounds as compared to all other forms of olive oil[10]. Apples undergo two step fermentation process for the production of ACV, which contains 5-6% acetic acid[12]. Phenolic compounds present in it have antioxidant and glucose lowering effects[11]. Apple cider vinegar has hepato-protective effect as phloretin present in it plays vital roles in the non-enzymatic protection against oxidative stress induced hepatic injury[13]. The aim of the current study was to determine and compare the hepato-protective effects of extra virgin olive oil and apple cider vinegar to identify which one of them may be more effective and potent in treating or reversing progression of hepatic damage in diabetics so that that these natural substances could be added as adjuvant therapy for diabetes.

MATERIAL AND METHODS

This experimental study, with a duration of 40 days, was conducted at the Animal House of Postgraduate Medical Institute, Lahore from May to June, 2021. Research was started after the official approval of synopsis by Ethical Committee of Postgraduate Medical Institute, Lahore (00-20-5-2019). Sample size was calculated using values of serum ALT levels in the following formula;

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

Animals

Forty adult healthy male Sprague Dawley rats of weight 180-200 gram were bought from local market of Lahore for this study. Rats showing signs of any ailment were excluded. Prior to the experimentation, the rats were given one week to be acclimatized. They were housed in labeled cages of appropriate size at room temperature (22-24ºC) with proper ventilation. They were fed on rat chow and water ad-libitum. After acclimatization, rats were divided into four groups by lottery method (10 rats in each group) i.e., Group I negative control (NC) group, Group II PC positive control, Group III extra virgin olive oil group (EVOO) and Group IV apple cider vinegar group (ACV).

Induction of Diabetes

Type-2 diabetes mellitus was induced at the start of study in all rats by administration of intraperitoneal injection of nicotinamide in a dose of 100mg/kg BW by 26 gauge needle followed by injection STZ (prepared in citrate buffer with pH of 4.5) in a dose of 55 mg/kg BW after 15 minutes, except the rats of NC group. Rats with blood glucose level >200mg/dL (checked by hand held gluco-meter, manufactured by SD Biosensor, Korea), 72 hours after injection of STZ were considered diabetic[14].

Dosage and Experimental Study Program

Both the control groups i.e., Group I (NC) and Group II (PC) were given 1ml/100gBW/day distilled water orally. Group III (EVOO) was given 1ml/100gBW/day extra virgin olive oil (Rafael Salgado, Spain)[15], Group IV (ACV) was given 2ml/kgBW/day diluted apple cider vinegar (Bragg Live Food Products) with distilled water in 1:5 for 4 weeks[16].

Measurement of Serum Biochemical Indexes

Light anesthesia with chloroform was done after overnight fast at the end of 4th week to collect samples using a syringe of 26-gauge needle through cardiac puncture and remains were disposed off in accordance with the ethical principles and guidelines laid down by World Medical Association (WMA) declaration of Helsinki. Four ml blood of each rat was collected and sampling tubes were kept straight in standing position for half an hour at room temperature and then centrifuged at 3000 rpm for 10 minutes for separation of serum. The serum was then poured into pre labeled serum holders. Liver enzymes (ALT, AST, ALP) were measured in serum samples by semi-automated chemistry analyzer by standard kinetic method. Data were entered and analyzed in SPSS version 26. Normality of distribution of data was checked by Shapiro Wilk test. The median (Inter Quartile Range) for quantitative variables were calculated. Differences in results among groups were compared and checked by applying Kruskal Wallis H test. Results among individual groups were analyzed by Mann Whitney U test.

RESULTS

A very highly significant difference in ALT levels (p= 0.000) as well as in ALP (p=0.000) levels were found among all the study groups, when comparison was done by Kruskal Wallis H test at the end of the study. Serum AST levels were significantly different among all the groups with p value 0.011, whereby the diabetic rats displayed highest values of ALT, AST and ALP (Table-1, Fig-1, Fig-2, Fig-3). Pair wise comparison was done by Mann Whitney U test that also revealed highest increase of serum ALT levels in PC group. Serum ALT levels of EVOO (p=0.026) & ACV (p<0.001) groups were significantly lower than PC group. On comparison with NC, EVOO group showed significant difference with p value of 0.006 while there was no significant difference of ALT levels of NC & ACV groups with p value of 0.649 (Table-2). Pair wise comparison of serum ALP levels revealed that serum ALP levels of EVOO & ACV groups were very highly significantly lower than PC i.e., p < 0.001. However, comparison of serum AST levels of both the treatment groups (EVOO & ACV) with PC group showed statistically non-significant difference with the p-values of 0.082 and 0.058 respectively (Table-2).

Groups		Alanine amino-transferase (U/L)	Aspartate amino-transferase (U/L)	Alkaline phospha tase (U/L)
Group-I (NC)	Median (IQR)	58.50 (49.75-66.25)	98.50 (83.00-107.25)	581.50 (493.75-740.00)
Group-II (PC)	Median (IQR)	92.00 (79.75-119.75)	117.50 (108.50-122.00)	1520.00 (1293.25-1783.75)
Group-III (EVOO)	Median (IQR)	76.00 (66.75-91.50)	112.50 (94.75-115.25)	850.00 (818.75-912.75)
Group-IV (ACV)	Median (IQR)	54.50 (48.50-64.75)	105.50 (98.50-113.50)	454.00 (350.75-474.75)
P value		**0.000*** **	**0.011***	**0.000*** **

Table-1: Comparison of serum liver enzymes (alanine amino-transferase, aspartate, aminotransferase and alkaline phosphatase) levels among all groups at the end of the study by Kruskal Wallis H test

*p value significant,
***p value very highly significant.

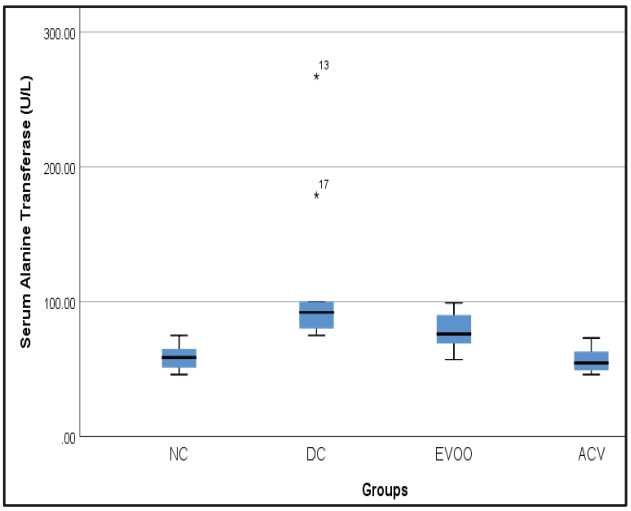

Fig-1: Serum Alanine aminotransferase levels {median (IQR)} ofrats in all groups at the end of 4 weeks study period.

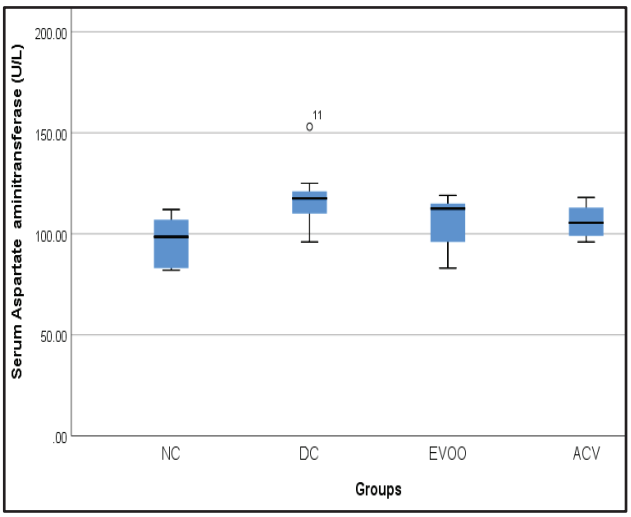

Fig-2: Serum Aspartate aminotransferase of levels {median (IQR)} of rats in all groups at the end of 4 weeks.

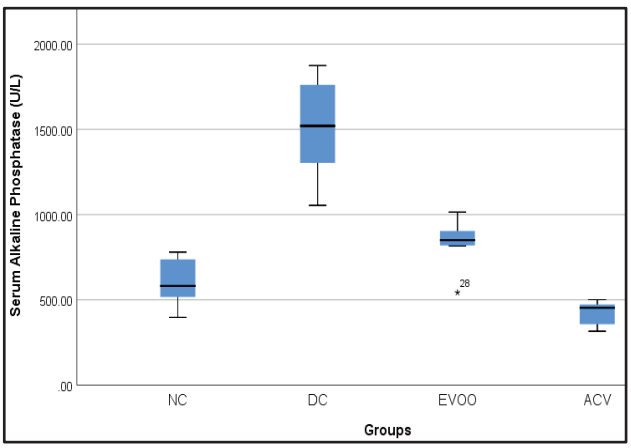

Fig-3: Serum Alkaline phosphatase levels {median (IQR)} of rats in all groups at the end of 4 weeks.

Groups		Alanine amino-transferase (U/L)	Aspartate amino-transferase (U/L)	Alkaline phosphatase (U/L)
Group I (NC)	Median (IQR)	58.50 (49.75-66.25) **²p<0.001*** **	98.50 (83.00-107.25) **²p=0.004** **	581.50 (493.75-740.00) **²p < 0.001*** **
Group II (PC)	Median (IQR)	92.00 (79.75-119.75)	117.50 (108.50-122.00)	1520.00 (1293.25-1783.75)
Group III (EVOO)	Median (IQR)	76.00 (66.75-91.50) **¹p=0.006** ** **²p=0.026***	112.50 (94.75-115.25) ¹p=0.064 ²p=0.082	850.00 (818.75-912.75) **¹p=0.001** ** **²p<0.001*** **
Group IV (ACV)	Median (IQR)	54.50 (48.50-64.75) ¹p=0.649 **²p<0.001*** **	105.50 (98.50-113.50) ¹p=0.074 ²p=0.058	454.00 (350.75-474.75) **¹p=0.005** ** **²p<0.001*** **

Table-2: Multiple comparison of serum liver enzymes (alanine aminotransferase, aminotransferase and alkaline phosphatase) levels among groups at the end of study by Mann Whitney U test

p¹= significance versus NC,
p²=significance versus PC
*p value significant,
**p value highly significant,
***p value very highly significant

DISCUSSION

Diabetes mellitus is considered as a great public health issue worldwide whose risk is increased by adopting sedentary lifestyle i.e., decreased physical activity and unhealthy dietary habits[2]. Hepatic damage is one of the complications of diabetes[9]. This study was designed to determine the hepatoprotective effect of EVOO and ACV in Type-2 diabetic rats.

Hepatic enzymes (ALT, AST and ALP) were estimated in serum samples of all rat groups at the termination of this study. The diabetic group developed a significant raise in serum ALT, AST and ALP) as compared to NC group. These findings are in accordance with previous literature which state that long term hyperglycemia induces oxidative stress, resulting in hepatic tissue injury[7,15]. Intake of EVOO has hepato-protective effect. Different bioactive molecules present in olives such as MUFA (oleic acid) and phenolic compounds i.e., HT and oleuropein turn on several signaling cascades in hepatic cells for prevention of oxidative stress, inflammation, endoplasmic reticulum stress, mitochondrial dysfunction and insulin insensitivity resulting in resolution or prevention of hepatic injury[15]

On comparison with PC group, both the treatment groups (EVOO and ACV) showed significant decrease in serum ALT levels (p values 0.026 and <0.001 respectively) and serum ALP levels (p values <0.001) in current study. However, reduction of serum AST levels was with p values 0.082 and 0.058 in EVOO and ACV groups respectively. Results of some previous studies confirm our findings. Kauka et al. attributed the improvement in liver enzymes by consumption of EVOO to the presence of hydroxytyrosol a phenolic component which prevents inflammation by inhibiting expression and activation of endothelial and intercellular adhesion molecules[17]. Amirnahavandirahbar and Nasirzadeh proved in their study that oleuropein (an important component of EVOO) causes decline in elevated liver enzymes as oleuropein inhibits cyclooxygenase-2 enzymeto bring about its anti-inflammatory action[18]. Liver enzymes are increased in diabetes due to hepatocellular inflammation. Polyphenols, present in olives have anti-inflammatory activity that's why they were reported to help in regulation of hepatic enzymes in diabetics[19,20]. However, contrary to our results, Sila et al. noted that consumption of olive oil has no effect on elevated liver enzymes (ALT, AST and ALP) in diabetic rats[21].

Apple cider vinegar also has hepatoprotective effect due to the presence of biologically active ingredients like flavonoids. Reduction of liver enzymes in our study by administration of ACV is in accordance with some previous studies. Halima et al., 2017 noted elevated hepatic biomarkers in diabetic group of their study, which were significantly decreased in the group treated by administration of ACV due to constructive effects of Quercetin (flavonoid present in ACV) on liver, suggesting that ACV plays an important role in improving hepatic function[22]. Bukhari et al., 2021 noted marked reduction in liver enzymes (ALT, AST, ALP) in high fat fed rats, by oral administration of ACV[23]. Bouazza et al., 2016 attributed improvement in liver functions and reduction in liver enzymes (ALT, AST, ALP) in serum by administration of ACV in their study to the presence of phenolic compounds in ACV[24]. Mohammad Ghasemi et al., 2018 indicated improvement in symptoms of NAFLD, reduction in grade of steatosis from moderate to mild along with improvement of liver enzymes levels by intake of ACV[25].

CONCLUSION

This study concludes that extra virgin olive oil and apple cider vinegar have a hepatoprotective effect in Type-2 diabetic rats as both were successful in

lowering hepatic enzymes. Hepatoprotective effect of apple cider vinegar was more pronounced.

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