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Effect of *Prunus Dulcis* & Álpha-Tocopherol in Ethanol Induced Dyslipidemia In Wistar Rats

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The study is aimed at assessing the effect of *Prunus dulcis* and alpha-tocopherol treatment against ethanol induced dyslipidemia in Wistar rats. 30 albino Wistar rats were selected based on the selection criteria and equally distributed into 5 groups – Control, ethanol, *Prunus dulcis*, alpha-tocopherol and combination of alpha-tocopherol +*Prunus dulcis* treated for 40 days. After the treatment for 40 days, all the animals were euthanized and a retro-orbital puncture was made to collect the blood samples for biochemical investigations. Obtained results were statistically analysed using ANOVA. Compared to ethanol group alpha tocopherol, *Prunus dulcis* and alpha tocopherol + *Prunus dulcis* treatment significantly decreased total cholesterol and triglycerides levels with p value <0.001. High density lipoprotein(66.31%) levels in the ethanol group were decreased compared to the control group and were significantly increased in other groups. Low density lipoprotein and Very low density lipoproteinlevels were higher in the ethanol group compared with the control group and were significantly reduced in other groups with p value <0.001.Results suggest that ethanol has an ill effect on the lipid profile. Treatment with *Prunus dulcius* and alpha-tocopherol both solely or in combination has produced beneficial effects against dyslipidemia.

Keywords: Dyslipidemia, *Prunus dulcius*, Alpha tocopherol.

Consumption of alcohol is a risk factor for various diseases leading to disability and death. Alcohol intake is a global burden of disease as stated by the world health organization. Consumption of 40grams of pure alcohol per day regularly and irregularly is defined as heavier drinking[1]which is associated with increased risk of major cardiovascular complications[2]. Small quantity of alcohol intake increases the high density lipoproteinis considered to exert cardio protective effects. Various studies have been performed to determine the quantity of alcohol consumption for the protective effects of cardiovascular disease. Of which,light, moderate and severe quantities were tested and have concluded different conflicting results. It is to understand that alcohol consumption is an addiction were when the person starts consuming would not keep their limit always. At

times would consume beyond their limit taking high quantity of alcohol.

World Health Organization recognized nuts as a portion of healthy heart food. It is known that the prevalence of heart disease is increasing worldwide and therefore consumption of nuts could decrease the risk related to heart disease as it is known that nuts like almonds and pistachios are complex food rich in mono and poly unsaturated fatty acids. They are capable of reducing blood cholesterol levels[5]. HumairaJamshed et al. suggested that regular dietary intake of almonds reduces the risk of cardiovascular disease by preventing obesity, diabetes, and dyslipidemia[6]. Various studies have been done in almonds with different doses.

Alpha-tocopherol is a form of vitamin E,alipid soluble antioxidant plays a major role in the prevention and radical scavenging. Vitamin E protects the lipids against oxidation and acts in the prevention of cardiovascular disease[7]. Alpha-tocopherol holds the various beneficial effects on lipid metabolism improves the lipid profile, also influences extra lipid effects on hemostasis, vasodilatation, adipose tissue function, anti-inflammatory, and antioxidative action. Alpha tocopherol acts as a defense against the peroxidation process of polyunsaturated fatty acids[8].

Almonds are *Prunes* that belong to the rose family, the Rosaceae. There are three varieties of almonds, all of which produce nuts, but only some are edible. Two varieties of almonds are grown commercially which can be categorized as sweet almonds and bitter almonds. Almonds possess pharmacological properties such as antistress, antioxidant, immunostimulant, laxative, and lipid-lowering. It is also considered as a useful food remedy for strengthening the muscles and in anemia. These edible almonds contain 12% dietary fibers, 6.3% sugar and 0.7% starch[9]. Many epidemiological studies and clinical trials have reported the benefits of almond consumption in various pathological health issues like diabetes mellitus, obesity, metabolic syndrome and hypertension. Almond kernels are rich in mono and polyunsaturated fatty acids, carbohydrates, proteins, vitamins (vitamin E and vitamin B), minerals (copper, calcium and magnesium), and various bioactive compounds (phytosterols, polyphenols,etc) and they are also used as a natural antioxidant and as an anti-inflammatory agent[10]. Almonds have been used in metabolic syndrome and proven to be effective in reducing the triglyceride levels, and mainly has effects in reducing the small and dense low density lipoproteins. Therefore this study is proposed to determine and compare the effects of *Prunus dulcius* and alpha-tocopherol in ethanol induced rats.

MATERIALS AND METHODS

30 Male albino Wistar rats weighing around 150 to 220 grams were purchased from laboratory animal facilities. The exclusion criteria for the study was infected and disabled animals. The study was initiated based on the Institutional animal ethical committee guidelines. 30 rats were equally divided into 5 different groups and were housed in a polypropylene cage and all animals were given regular food pellets and drinking water in natural dark/light scheduled at 25°C±2°C for 47 days with the inclusion of 7 days of an acclimation period. The materials used in this study wereEthanol (99%), Normal saline, alpha-tocopherol and Almond oil. All the group rats were treated through oral gavage for 40 days. Group A being the Control group was treated with 0.5ml of dimethyl sulfoxide. Group B was given4g/Kg bodyweight of ethanol. Group C is a combination of ethanol and alpha-tocopherol, 180mg/kg bodyweight of alpha-tocopherol was mixed with 0.5% dimethyl sulfoxide and 4g/kg body weight of ethanol was given. Group D is a combination of ethanol and *Prunus dulcius*. 350mg/kg bodyweight of *Prunus dulcius* was mixed with 0.5 % of dimethyl sulfoxide and ethanol 4g/kg body weight. Group E is combination of alpha-tocopherol, *Prunus dulcis* and ethanol, the ethanol dosage was 4g/kg bodyweight, 180mg/kg bodyweight of alpha-tocopherol was diluted with 0.5 % of dimethyl sulfoxide and 350mg/kg body weight of *Prunus dulcius* was diluted with 0.5 % of dimethyl sulfoxide.

After the treatment for 40 days all the animals were euthanized and a retro-orbital puncture was made to collect the blood samples for biochemical investigations.

RESULTS

Table 1 reveals that high density lipoprotein levels in the ethanol group is decreased to 66.31% compared to the control group. When compared to the ethanol group high density lipoprotein level increased to 51.69% inalpha-tocopherol group, 51.87%in *Prunus dulcis* group and 59% in combination group of alpha- tocopherol + *Prunus dulcis.*

The low density lipoprotein levels were 47.64%higher in ethanol group compared with the control group. Low density lipoprotein levels were markedly decreased to 26.63% in alpha tocopherol group,27.86% in*Prunus dulcis* group and 24.60%in combination group of alpha-tocopherol + *Prunus dulcis* compared with the ethanol group

The very low density lipoprotein level in the ethanol group was increased46.49% compared to the control group. Whereas other groups showed a decrease in very low density lipoprotein compared to the ethanol group that is 12.92% inalpha-tocopherol group,14.5% in*Prunus dulcis* group and 11.29%in combination group of alpha-tocopherol + *Prunus dulcis*.

Table 2 shows, Increase in serum total cholesterol and triglycerides due to ethanol consumption. Compared to ethanol group alpha tocopherol, *Prunus dulcis* and alpha- tocopherol + *Prunus dulcis* treatment significantly decreased total cholesterol and triglycerides levels. Total cholesterol levels compared to ethanol group showed 12.19% reduction in the alpha-tocopherol group, 13.22% in*Prunus dulcis*group,9.32% reduction in thecombination ofalpha-tocopherol + *Prunus dulcis*group. Thetriglyceride level in the alpha-tocopherol group was 13% reduced, 14.49% in *Prunus dulcis* group and11.27% reduction in the combination ofalpha-tocopherol + *Prunus dulcis*compared to ethanol group respectively.

DISCUSSION

Alcohol intake is a public health concern and burden worldwide as it elicits deleterious effects on multiple organs and serious injury to health includes neural problems, reproductive issues, cardiovascular diseases, diabetes, suppresses the immune system and other diseases. Ethanol is metabolized by two pathways oxidative and non-oxidative. Non-oxidative metabolism takes place in the pancreas which involves the process of esterification reactions of fatty acids and results in producing the fatty acid ethyl esters, these ethyl esters increase the fragility of lysosomal membrane and oxidative stress. Cholesterol esters mediate the instability of the lysosomal membrane and further, the transesterification process synthesizes the cholesterol ester and produces the free fatty acids by hydrolysis[3].It has been stated that low density lipoproteins are of multiple classes includes dense, small, lipid depleted particles to buoyant, large, cholesterol-enriched particles. Of which small dense LDL particles are more associated and contributing factors for coronary vascular disease. Coronary vascular disease is a major risk factor caused by lipoproteins, the atherogenicdyslipidemia is a major complication defined from high plasma concentration of low high density lipoproteins, triglycerides, and small dense particles of low density lipoproteins. Dyslipidemia is been a component of various related diseases like metabolic syndrome includes abdominal obesity, high blood pressure and dysglycemia. Studies have

Table 1. Comparison of Very Low, low and High density lipoprotein levels

Parameters (mg/dl)	Control group	Ethanol	Alpha tocopherol	*Prunus Dulcius*	Alpha tocopherol + *Prunus dulcius*	F value	P value
HDL	46.65±0.806	28.05±1.331	42.55±1.228	42.60±0.575	44.63±1.303	45.677	<0.001
LDL	83.14±1.983	122.75±2.778	90.05±1.550	88.54±0.640	92.55±1.225	76.885	<0.001
VLDL	15.53±0.414	22.75±0.303	19.81±0.442	19.45±0.345	20.18±0.372	46.885	<0.001

HDL – High density lipoprotein, LDL – Low density lipoprotein, VLDL – Very low density lipoprotein, The data were expressed as Mean ± SEM and analysed by one way ANOVA and multiple comparison test with Student Newman Keul's method.

Table 2. Comparison of total cholesterol and triglycerides

Parameters (mg/dl)	Control group	Ethanol	Alpha tocopherol	*Prunus Dulcius*	Alpha tocopherol + *Prunus dulcius*	F value	P value
Total Cholesterol	145.31±1.486	173.56±1.504	152.40±1.383	150.61±0.710	157.38±0.799	77.512	<0.001
Triglycerides	77.63±2.123	113.83±1.529	99.00±2.181	97.33±1.709	101.00±1.892	46.658	<0.001

TC – Total Cholesterol and TG – Triglyceride levelsThe data were expressed as Mean ± SEM and analysed by one way ANOVA and multiple comparison test with Student Newman Keul's method

proven that low fat and high carbohydrate diet increase the small dense low density lipoprotein levels and plasma triglyceride levels. Diet with reduced carbohydrates and constant protein intake has produced better results[4]. Physical inactivity, diabetes and dyslipidemia are major risk factors for cardiovascular events and therefore need both pharmacological and non-pharmacological management[16].

Exposure to alcohol has an impact on lipolysis, lipid profile and lipid peroxidation process and results in alcoholic hypercholesterolemia, hypertriglyceridemia. Therefore it is very important to manage the ethanol induced changes in lipid peroxidation process and metabolism. Therefore this study proposed a natural treatment with *Prunus dulcius* and alpha-tocopherol for ethanol induced dyslipidemia[11].

Afaf A Tarmoos et al., (2019) examined the effects of almond suspension on biochemical parameters of mice after inducing hyperlipidaemia with excess cholesterol in the diet for 60 days and observed the significant decrease in low density lipoprotein, very low density lipoprotein levels and triglycerides and total cholesterol levels and a significant increase in high density lipoprotein levels with a dosage of 857, 1128, and 1428 mg/kg sweet almond suspension[12]. The study proved the effects of almond as lipid lowering agent. In this study HDL levels in the ethanol treated groups were markedly decreased when compared to the control group. Whereas the HDL levels in the alpha-tocopherol group, *Prunus dulcis* group and combination group of alpha-tocopherol + *Prunus dulcis* were increased compared with the ethanol group.

Ghada Z A Solimancompared the effects of pistachio and almonds on the lipid profile in hypercholesterolemic rats and demonstrated that dietary supplements with both the nuts had a greater effect in decreasing total cholesterol, low density and very low density lipoproteins and triglycerides[5]. In this study LDL and VLDL levels of the ethanol group were significantly high compared to the control. The levels of LDL and VLDL in the alpha-tocopherol group, *Prunus dulcis* group and combination group of alpha-tocopherol + *Prunus dulcis* group were markedly decreased compared with the ethanol group.

Two randomized crossover study by

Chen and collegues directly investigated the effect of almonds on the risk of coronary heart disease among 27 hyperlipidemic men and postmenopausal women. After intervention, there was a significant improvement in the lipid profile in the almond intervention group. In particular, it was estimated that every 7g almonds daily can reduce low density lipoprotein by 1% therefore reducing 2% risk for coronary heart disease[13]. In this study, in rats treated with ethanol show the serum total cholesterol levels appear significant rise in comparison with the control group. The combination of ethanol with alpha-tocopherol (Group III), *Prunus dulcis* (Group IV), and combination of alpha-tocopherol + *Prunus dulcis* (Group V) showed a distinct reduction of the total cholesterol and triglyceride levels.

Etim O E et al.,have studied the effects of sweet almond extract in serum lipids in diabetes induced rats and concluded that complication of diabetes mellitus is associated with lipid metabolism which increases lipid peroxide levels in diabetes and concluded that sweet almond extract is effective in protecting and reducing the damage to the pancreas and prevents the complications of diabetes[14]. Mona Anwar et al., have determined the effects of almond oil and extracted diosmin in diabetic rats and proved that almond oil has more prophylactic effects in endothelial dysfunction of diabetic rats[15]. Whereas, this study determined the effects of *Prunus dulcius* and alpha tocopherol to be effective in managing the dyslipidemiain ethanol induced toxicity

CONCLUSION

Results suggest that ethanol has an ill effect on the lipid profile. Body weight ratio could be monitored to determine any significant difference between body weight ratio and lipid profile. Treatment with *Prunus dulcius* and alpha-tocopherol both solely or in combination has produced beneficial effects against dyslipidemia.

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Conflict of interest

None.

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