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A Prospective Study to Evaluate Daily Moderate Consumption of Ethanol on Oxidative Stress Markers of Diabetes Induced *Wistar* Strain Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors STP and SE designed the study. Author STP wrote the protocol. Authors SGK, RA and AS managed the literature searches. Authors STP and SPS performed experiment and analyzed the results. Author STP wrote the first draft of the manuscript. Author SE verified the manuscript. All authors read the final manuscript.

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ABSTRACT

Objective: The objective of this study is to compare the antioxidant enzyme superoxide dismutase (SOD) level and lipid peroxidation product malondialdehyde (MDA) in ethanol treated non diabetic and diabetic rats.

Methods: A total of 24 male Wistar *albino* rats were grouped as control (n=6), diabetic control (n=6), ethanol treated control (n=6) and ethanol treated test (n=6) groups. Total duration of this experiment was 30 days. Diabetes mellitus was induced in rats by a single intraperitoneal dose of streptozotocin at 40 mg/kg dissolved in 0.1 M cold citrate buffer. After the confirmation of diabetes

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in streptozotocin administered rat groups, on the 10th day of the experiment healthy control and diabetic control groups were orally treated with (1.0 mL/Kg body weight) drinking water while diabetic and non-diabetic ethanol control rat groups were orally treated with (1.0 mL/Kg body weight) 6.0% ethanol. On the 20th day, treatment was stopped and restarted on 25th day of the experiment.

Results: At the end of the experiment, the body weight of the healthy rat groups gradually increased (251±20.77g) when compared with diabetic groups (162±07.48 g for diabetic control and 176±24.78 g for ethanol treated diabetic groups). The ethanol treated diabetic groups showed a significantly reduced blood glucose level ($P < 0.01$) than the diabetic control groups. The moderate amount of ethanol treated diabetic rats showed normal SOD (3.928 Unit mg/min) and decreased MDA (ethanol treated diabetic rats showed 1.0156 nmol/mg protein) than the diabetic rats (1.7638 nmol/mg protein).

Conclusion: This study indicates that daily low consumption of alcohol may reduce the risk of oxidative stress and try to normalize the antioxidant status in diabetes rats.

Keywords: Diabetes mellitus; streptozotocin; ethanol; liver; superoxide dismutase; malondialdehyde.

1. INTRODUCTION

Oxidative stress can be defined as a state of imbalance between the production of free radicals and the ability of the body to counteract it. It also helps in many physiological process by means of intercellular signal transduction. Under normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and antioxidant defense systems [1]. In a clinical view point, oxidative stress reflected the pathological features of different diseases especially lifestyle related diseases [2]. Cellular disturbances may be due to the effect of external or internal factors. It adversely affects the normal redox status of a typical cell by the production of peroxides and free radicals that can damage all components of the cell, including proteins, lipids and DNA. As per the Halder study report, oxidative stress is the imbalance between the generation of reactive oxygen species and the body defense mechanisms [3]. In recent times, there is an increased incidence of lifestyle diseases which may also be directly or indirectly associated with oxidative free radicals. The major lifestyle diseases are neurodegenerative disorders, mutagenesis, heart and blood vessel disorders, chronic fatigue syndrome, diabetes mellitus, kidney diseases etc.

Lifestyle disease is also called diseases of longevity or diseases of civilization interchangeably. In fact, that origin of the disease is anthropogenic. Technological advancements, industrialization, daily habits of people and inappropriate relationship of people with their environment etc., are the major causes of this situation. In addition to these, several other factors contributed to the origin of lifestyle diseases. Common causes include population, bad diet options, lack of adequate exercise, alcoholism, environmental conditions like work environments, stress etc. In humans, alcohol consumption is a serious health hazard. Alcohol consumption may alter the normal functioning of different organs. Liver is a major organ susceptible to this effect. Liver damage may be due to the consequences of chronic alcoholism. It may be progressively associated fatty liver and further hepatic cirrhosis. Perhaps it may happen from the generation of oxygen free radicals or other reactive species which are derived from the alcohol induced metabolism. According to Cahill report, acute and chronic ethanol exposure lead to increased mitochondrial production of reactive oxygen species [4]. An increased oxidative stress and free radical - mediated tissue damage occur in alcoholic liver [5]. Bosch has reported that ingestion of alcohol triggers decreased cellular antioxidants due to the effect of abnormal oxidative stress [6]. From the above data, it is clear that a relationship exists between free radical mediating tissue damage and effect of alcohol consumption. The evidences from the review suggested that the free radicals are the cornerstone of alcoholism and lifestyle diseases.

Diabetes mellitus is one of the major health problems in the above categories. According to World Health Organization diabetes mellitus is the eighth leading cause of death in world population [7]. In 2014, the International Diabetes Federation (IDF) estimated that diabetes resulted in 4.9 million deaths [8]. There are various causative factors for diabetes mellitus, the major attributing factor are type of food, eating pattern, smoking, alcohol consumption, stress etc., which are together

called modern living habits. Diabetes mellitus is strongly influenced by lifestyle factors especially alcohol consumption. An earlier study has been reported that there is a relation between alcohol intake and risk of type II diabetes [9]. Several lifestyle factors affect the incidence of type II diabetes, and alcoholism is one of the factors. Obesity and weight gain dramatically increases the risk of diabetes mellitus. On the other hand, Ajani's study reported that light to moderate drinking of alcohol may protect against development of diabetes [10]. Another study of Castelnuovo revealed that moderate alcohol consumption correlates with modification of several vascular and biochemical factors that have potential cardio-protective benefits [11].

Diabetes mellitus is a heterogenous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism that ultimately cause the formation of free radicals. These substances are generally unstable and very reactive, such as reactive oxygen species, reactive nitrogen species and reactive chlorine species. Reactive oxygen species (ROS) are superoxide anion, hydroxyl radical and hydrogen peroxide [12]. Generally, ROS are produced in the human body and plays an important role in the biochemical reactions including detoxification, chemical signalling and immune function [13]. The cellular sources of free radical reactions include oxidative phosphorylation, glucose autoxidation [14], nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and other enzymes such as xanthine oxidase, lipoxygenase, cytochrome P450 monooxygenases, and nitric oxide synthase [15]. After ROS is formed, it in turn disturbed the antioxidant defence systems (free radical scavengers or ROS detoxifying enzymes). If ROS are not detoxified, cellular components are damaged and signalling pathways are altered and results in complications in diabetes mellitus.

Oxidative stress is highly correlated with lifestyle metabolic diseases especially diabetes mellitus. The possible source of oxidative stress in diabetes includes shifts in redox balance resulting from altered carbohydrate and lipid metabolism [16], hyperglycaemia, hypertension, obesity etc., which are the major changes of diabetic people. Alcohol consumption is not a major issue of diabetes but regular consumption raises deleterious effect to different organs and moreover it is metabolized in the liver. Alcohol induced tissue disturbances may be generating oxygen free radicals during its oxidation [17]. Effects of alcohol differ according to species, dose and whether it is used in fasting or fed state. A study of Das and Vasudevan observed that not only kidney but also liver was affected due to chronic alcohol consumption [18].

Liver damage in both diabetes and chronic alcoholics may cause to release xanthine oxidase into the blood. Xanthine oxidase is a form of xanthine oxido-reductase, an enzyme that generates reactive oxygen species. This enzyme catalyzes the oxidation of hypoxanthine to uric acid. Under normal physiological conditions xanthine oxidase acts as a dehydrogenase enzyme. It removes hydrogen from xanthine or hypoxanthine and attaches it to NAD, thereby generating NADH. However, abnormal conditions like the disruption of blood flow to a particular tissue, xanthine dehydrogenase is converted to a reactive oxygen species producing an oxidized form of it. Desco and associates reported that xanthine oxidase (a superoxide generating enzyme) is increased in plasma and liver of diabetic rats [19]. Alcohol consumption also may promote the conversion of xanthine dehydrogenase to xanthine oxidase [20], which can generate ROS, thereby enhancing oxidative stress. Consequently, enhanced oxidative stress can lead to depressed antioxidant status in alcoholics. Some of daily habits like smoking, over eating, alcoholism etc., are closely associated with oxidative stress. The fact is that the level of antioxidant enzymes critically influence the susceptibility of oxidative stress in various tissues. Kakkar reported that the increase in the level of oxygen free radicals in diabetes could be decreased by the destruction of non enzymatic and enzymatic antioxidants such as catalase, glutathione peroxidase and superoxide dismutase [21]. The integrated antioxidant system which is scavenging reactive oxygen species is derived from free radicals. The accurate assessment of free radical scavengers were necessary for investigation of various pathological conditions [22]. This kind of assessment will be helpful to know the role of oxidative stress and how it acts against antioxidants in diabetic conditions. Alcohol consumption also induces some changes in body antioxidant status. However, this present study also evaluated the activity of antioxidant in alcohol treated diabetic and non-diabetic rats. Thus the aim of this study was to evaluate the effect of alcohol on antioxidant enzyme, superoxide dismutase (SOD) and lipid peroxidation status of diabetic and non-diabetic alcohol treated rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Streptozotocin was purchased from the Sigma chemicals Co. USA. The required percentage of ethanol (6%) was made in the laboratory. Ethyl alcohol was procured from Excise Range office, Deputy Commissioner of Excise, Wayanad, Government of Kerala.

2.2 Animals

Male *Wistar* albino rats were selected for the study. All the animals weighed about 160-220 grams and approximately 60 days old. The rats were maintained in the animal house of the Department of Life Sciences (Reg # 426/02/CPCSEA). Rats were housed in clean polypropylene cages of dimension 29 cm x 22 cm x 14 cm with wire mesh lid with sterilized paper strips as bedding materials. The rats were kept under standard conditions (maintained at a temperature of 23ºC - 25ºC), with standard pellet diet and water *ad libitum* throughout the experimental period. All the studies were conducted strictly in accordance with the approved guidelines of the Institutional Animal Ethics Committee regulated by the committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social justice and Empowerment, Government of India.

2.3 Acute Oral Toxicity Study

Healthy 3 adult male *Wistar* albino rats were orally fed with different doses of ethanol to study oral toxicity and the normal behaviour of rats. Each of the rat was fed with 6% ethanol at different doses as 1 mL/Kg, 2.5 mL/Kg and 5 mL/Kg. There was no lethality in animals with the above mentioned doses of ethanol. The minimum dosage (1.0 mL/Kg body weight) of ethanol was selected for this study.

2.4 Induction of Diabetes Mellitus

Experimental animal diabetic model is induced by streptozotocin (STZ). The rats were made diabetic with a single intraperitoneal injection of freshly prepared streptozotocin in 0.1 M cold citrate buffer (pH- 4.5) at a dosage of 40 mg/Kg body weight. After the drug administration, 20% glucose solution was orally administered through oral gavage for reducing the risk associated with fasting hypoglycaemic condition. After a 4 days' observation, blood glucose level was checked and rats with fasting blood glucose level between 200-250 mg/dL were considered as diabetic animals. Then the rats included in the study are grouped as:

Group I: Healthy control rats treated with drinking water at 1.0 mL/Kg body weight.
Group II: Diabetic control rats treated with drinking water at 1.0 mL/Kg body weight.
Group III: Ethanol control rats treated with 6% 1.0 mL ethanol/ Kg body weight.
Group IV: Diabetic rats treated with 6% 1.0 mL ethanol/Kg body weight.

2.5 Analytical Procedures

Body weight of the experimental rats was checked every day and the blood glucose level was checked in a five day interval. From the tenth day of the experiment, 6% ethanol was orally administered for group III (ethanol control) and IV (diabetic rats treated with ethanol or diabetic test) using an orogastric neonatal feeding tube. On the 20th day of the experiment, there was a significant reduction in the blood glucose level in all the experimental animals except the diabetic control group. So the treatment was stopped for 5 days. On the 25th day, there was an elevation in the blood glucose level. Therefore, the treatment was again continued upto the 30th day of the experiment. On the 30th day of the experiment, blood glucose level was checked using a glucometer. After that, the rats were sacrificed by deep anaesthetization. Then the liver tissue was isolated for biochemical estimation of oxidative markers related with both diabetes mellitus and daily low consumption of ethanol. The present study analysed activities of the superoxide dismutase, lipid peroxidation product malondialdehyde and tissue protein.

2.5.1 Biochemical assay of oxidative stress markers

2.5.1.1 Super oxide dismutase assay

Super oxide dismutase was estimated according to the method described by Kono [23]. The reaction mixture contained 1.3 mL of solution A (0.1 mM EDTA containing 50 mM Na_2CO_3, pH - 10.5), 0.5 mL of solution B (90 µM NBT (Nitro Blue Tetra zolium dye) and 0.1 mL of solution C

(0.6% Triton X-100 in solution A). Reaction was initiated by the addition of 0.1 mL of solution D (20 mM Hydroxylamine hydrochloride, pH - 6.0) to the reaction mixture and the rate of nitro blue tetrazolium reduction in the absence of the enzyme source was recorded for about 30 seconds. Following this, small aliquots of supernatant (0.1 mL of tissue sample) were added to the test cuvette as well as reference cuvette, which did not contain hydroxylamine hydrochloride (solution D). Finally, percentage of inhibition in the rate of NBT reduction was noted. One enzyme unit was expressed as inverse of the amount of protein (mg) required for inhibiting the reduction rate of NBT by 50% in one minute [24].

2.5.1.2 Lipid peroxidation (LPO) assay

Lipid peroxidation was measured according to the method of Wills [25]. Malondialdehyde is one of the final products of polyunsaturated fatty acids peroxidation in the cells. Malondialdehyde level is commonly known as a marker of oxidative stress.

2.5.1.2.1 Malondialdehyde assay

Lipid peroxidation product MDA formation was assayed by thiobarbituric reactive substance formation method [26]. The tissue homogenate of both liver and pancreas were prepared in 0.1 M tris HCl buffer (pH-7.5), 1 ml of homogenate was combined with 2 ml of the TCA-TBA-HCl reagent and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 15 minutes. The absorbance of the sample was read at 535 nm against a blank that contained no tissue homogenate by JASCO V-630 Spectrophotometer (serial No. C395561148), Japan. The extinction coefficient of malondialdehyde is 1.56 X 10^5 $m^{-1}cm^{-1}$ and the results were expressed as nanomoles of MDA per mg protein.

2.5.1.3 Protein estimation

Protein content in tissue samples was estimated by the method of Lowry [27] using Bovine Serum Albumin (BSA) as a standard of protein.

2.6 Statistical Analysis

Experimental data were expressed as mean ± standard deviation (SD). Statistical significance was analysed by one-way ANOVA followed by Fisher's least significant difference (LSD) using Statistical Packages for Social Sciences (SPSS) version 16. Statistical probability of $P< 0.01$ or 0.05 was considered to be significant.

3. RESULTS AND DISCUSSION

Diabetes mellitus is one of the chronic diseases recorded world wide. It is a lifestyle modifying metabolic disorder mainly by hyperglycaemia, which is due to defect in insulin secretion, function and or both [28]. The current study focussed to compare the effect of daily consumption of low percentage alcohol on body weight, fasting blood glucose level, oxidative scavenging enzyme superoxide dismutase and lipid peroxidation product malondialdehyde level in diabetic and non-diabetic rats. Oxidative stress is caused by the overload of oxidants like reactive oxygen species. It impairs cellular physiology and leads to diseases. A reduction of antioxidants in diabetic condition due to increased oxidative stress. Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. Superoxide dismutase is a class of closely related enzymes that catalyse the breakdown of the superoxide anion. SOD enzymes are present in almost all aerobic cells and in extracellular fluid. The results mentioned in the following tables and figures were compared in each of the groups.

3.1 Effect on Body Weight

Oxidative stress is generally a cause of weight loss. According to Ogugua's report diabetic condition can adversely affect body weight [29].

Based on the data shown in Table 1 it has been revealed that there was a significant increase of the body weight in diabetic and non-diabetic rats when treated with a daily low consumption of ethanol. On the first day of the experiment, the body weight (mean and standard deviations or SD) of healthy control (n=6) group was 203±7.00 g, diabetic control (n=6) 201±11.43 g, ethanol treated non diabetic control (n=6) 151±23.55 g and ethanol treated diabetic rats (n=6) 170±24.86 g. At the end of the experiment, there was a slight variation of body weight in both diabetic and non-diabetic rats. The body weight was expressed in mean ± SD and the values

were 251±20.77 g, 162±7.48 g, 191±14.89 g and 176±24.78 g for healthy control, diabetic control, ethanol treated non-diabetic and diabetic groups respectively. From the result it was observed that there was a progressive increase in the body weight of healthy control rats, while diabetic rats showed a decrease in body weight when compared with ethanol treated diabetic rats. These data were analysed with multiple comparison using post hoc, where the *P* -value was less than 0.01 ($P<0.01$). So the results were statistically significance at 1% level. Shanmugam reported a significant reduction in body weight in alcohol treated diabetic rats compared to diabetic rats [30].

3.2 Effect on Blood Glucose Level

During diabetes there is an increased production of reduced sugars through glycolysis or polyol pathway initiated from the reactive oxygen species.

A comparison of blood glucose level in various groups of rats are summarized in Fig. 1. The mean (Mean ± SD) blood glucose levels at the first day of experiment were 115±24.41 mg/dL, 124±19.44 mg/dL, 105±22.08 mg/dL and 98.33±10.61 mg/dL for healthy control (n=6), diabetic control (n=6), ethanol control (n=6) and ethanol test (n=6) respectively. After inducing diabetes (on the 10^{th} day of experiment) the blood glucose level of streptozotocin groups were 449±139 mg/dL and 371±94.24 mg/dL. After a continuous 10 day treatment, there was a significant reduction in blood glucose level in 6% ethanol treated diabetic rats (107±27.50 mg/dL) when compared with diabetic control rats (374±70.06 mg/dL). On the 30^{th} day of the experiment we observed that the blood glucose level of healthy control was 116±16.70 mg/dL, diabetic control was 339±85.06 mg/dL, ethanol control was 119±35.29 mg/dL and 105±12.36 mg/dL in ethanol treated diabetic rats. The above data were analysed with multiple comparison of each of the groups obtained and the p-value is less than 0.01 ($p<0.01$). This study shows statistical significance between the groups at 1% level. Issabeagloo reported that mild consumption of alcohol helps to decrease the blood glucose level significantly [31]. From the result of this study, we conclude that moderate daily consumption of alcohol (6%) may have a tendency to decrease blood glucose level in hyperglycaemic conditions. Van de Wiel's report substantiate that hepatic gluconeogenesis is decreased by consumption of alcohol [32]. Another study suggests that moderate consumption of alcohol has an ameliorative effect on diabetes mellitus [33].

3.3 Effect on Antioxidant Enzymes

3.3.1 Liver superoxide dismutase

The possible sources of oxidative stress in diabetes might include auto-oxidation of glucose, shifts in redox balances, decreased concentrations of antioxidants like reduced glutathione (GSH) and vitamins, impairment in antioxidant defence enzymes such as SOD and catalase. It means that during diabetes or stressful conditions, free radicals may adversely affect normal functions of cells. This study focussed the activity of superoxide dismutase in the liver of ethanol treated diabetic and non-diabetic rats.

The enzymic antioxidant defense systems are the natural protector against ROS originated from oxidative stress. The antioxidant defense enzyme, SOD is involved in the rapid dismutation of superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2) [34]. The activities of total SOD in different groups are shown in Fig. 2. The mean

Table 1. Comparison of body weight (Mean±SD) in different groups

Sl no.	Groups	1^{st} day	5^{st} day	10^{th} day	15^{th} day	20^{th} day	25^{th} day	30^{th} day
1.	Healthy control	203±07.00	203±07.00	214±16.54	228±22.54	241±24.48	249±19.21	251±20.77
2.	Diabetic control	201±11.43	201±11.43	176±27.12	172±28.05	166±26.50	167±27.06	162±07.48
3.	Ethanol control	151±23.55	151±23.55	163±19.82	173±14.62	173±11.70	182±12.30	190±14.89
4.	Ethanol test	170±24.86	170±24.86	164±14.31	155±5.71	166±10.65	172±14.96	176±24.78

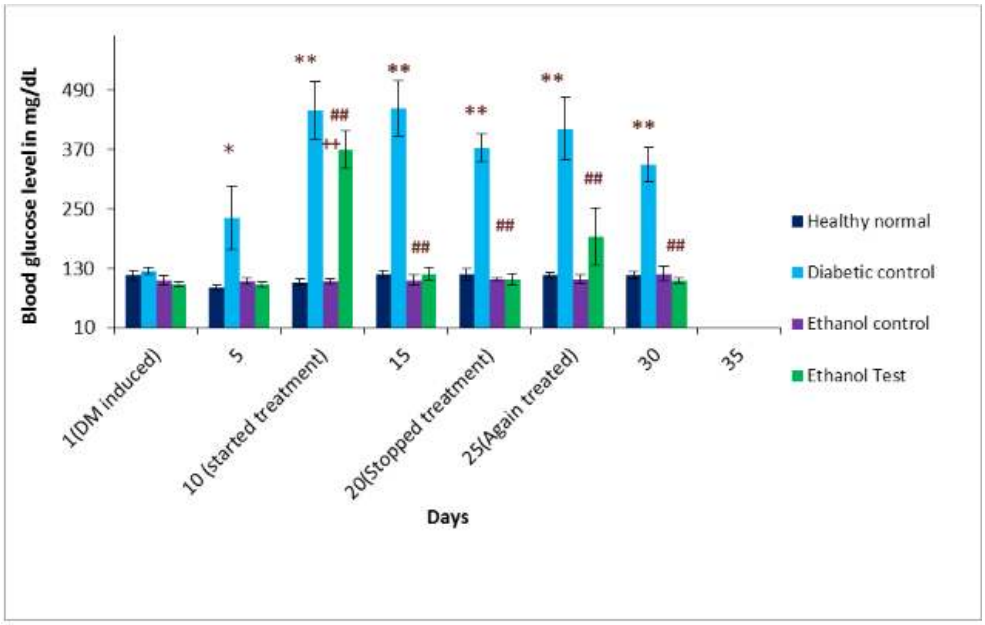

Fig. 1. Showing the comparison of blood glucose level in various groups

** - Comparison between healthy control and diabetic control*
+ - Comparison between ethanol control and ethanol test
 # - Comparison between ethanol test and diabetic control
** - Significant at 5% level*
*** - Significant at 1% level*
##- Significance at 1% level

values of hepatic SOD were 5.406±2.16, 1.700±0.534, 4.628±1.19 and 3.928±1.81 Units/mg of protein for healthy control (n=6), diabetic control (n=6), ethanol treated non-diabetic (n=6) and diabetic rats (n=6) respectively. From the results, SOD level of healthy control and ethanol treated non-diabetic was approximately equal but the activity of hepatic SOD was reduced in diabetic rats. In addition to these this study also correlated alcohol consumption with the activity of SOD. Hence the present study shows that there is significant reduction in the activity of the hepatic SOD in diabetes group when compared with ethanol treated non-diabetic and diabetic group. Several studies have been reported that SOD activity was decreased with alcohol consumption in liver, heart, brain, kidney, muscle and serum of rats [35-36]. This study showed that there is a significant difference between diabetic control and others group at 1% ($P < 0.01$) level and diabetic control and ethanol treated diabetes group at ($P < 0.05$) 5% level.

3.3.2 Liver malondialdehyde

Alcohol consumption in humans is a serious health issue because liver is the major organ most susceptible to it. Direct consumption of alcohol is also associated with elevated level of lipid peroxidation. It results with a number of changes in cell function and the oxidant-antioxidant system [37]. Lipid peroxidation of unsaturated fatty acids is frequently used as an indicator of oxidative stress and subsequent oxidative damage. Malondialdehyde is a highly reactive compound resulting from lipid peroxidation of poly unsturated fatty acids. It is one of the markers of oxidative stress. Streptozotocin induces diabetes mellitus by destroying the pancreatic beta (β) cells, possibly through generating excess reactive oxygen species [38]. A report of Pavana over production of reactive oxygen species and insufficient antioxidant defense mechanism is well documented in diabetic patients as well as in experimental diabetes mellitus [39]. Sudden release of reactive oxygen species in diabetes can lead to very serious complications including cardiovascular disease, liver and kidney failure. Several studies in humans and animal models, using thiobarbituric acid reactive substances assay have shown increased lipid peroxidation in membranes and lipoproteins in the diabetic state [40]. A significant increase of MDA was found to be obesity mediated in oxidative stress [41].

Therefore this prospective study also evaluated the hepatic malondialdehyde level in ethanol treated non-diabetic and diabetic rats.

Changes in lipid peroxidation product MDA in hepatic tissue of different groups are presented in Fig. 3. Within the diabetes group there was an

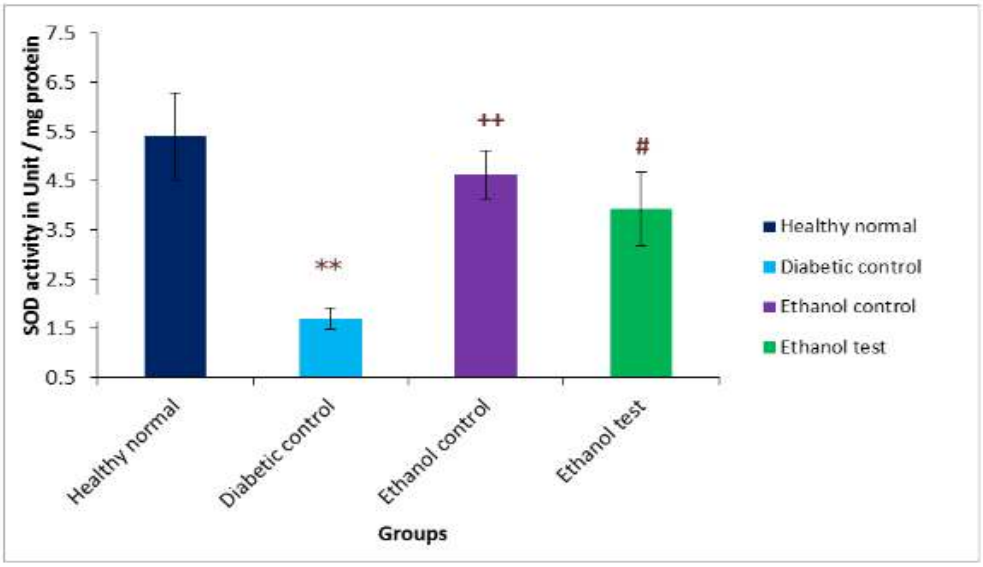

Fig. 2. Showing the comparison of superoxide dismutase activity in various groups

**- Comparison between healthy control and diabetic control*
+ - Comparison between ethanol control and ethanol test
 # - Comparison between ethanol test and diabetic control
 # - Significant at 5% level
*** - Significant at 1% level*
++- Significant at 1% level

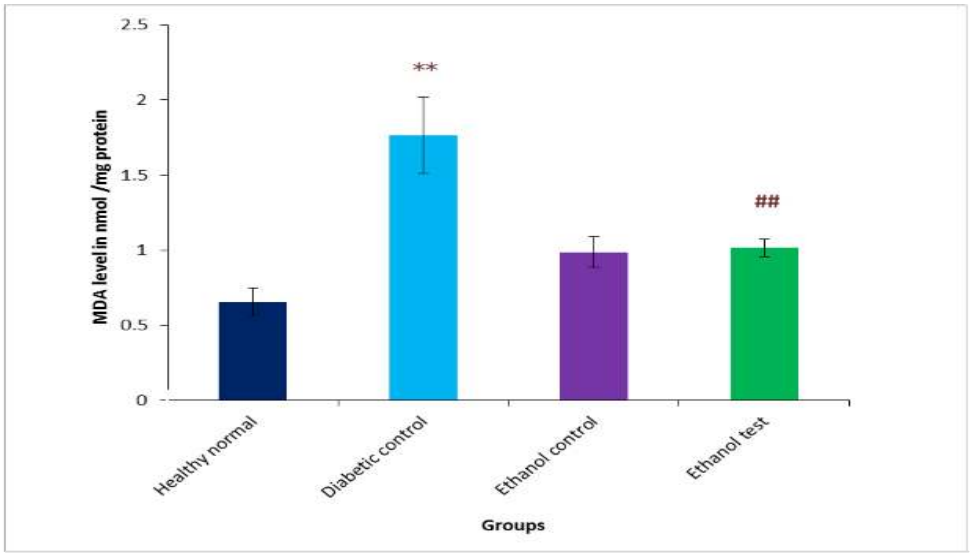

Fig. 3. Comparison of lipid peroxidation product MDA in liver of different groups

***- Comparison between healthy control and diabetic control*
#- - Comparison between ethanol test and diabetic control
*** - Significace at 1% level*
- Significace at 1% level

increase in the hepatic MDA compared with healthy control group. The mean values of MDA were 0.6572±0.22 nmol/mg protein for healthy control, 1.7638±0.61 nmol/mg protein for diabetic control, 0.9879±0.25 nmol/mg protein for ethanol control and 1.0156±0.14 nmol/mg protein for ethanol test. When healthy control group was compared with ethanol control rat, a significant change was observed in hepatic MDA but this study did not show statistical significance. Simultaneously, there was a significant increase of hepatic MDA in diabetic control group compared with that of ethanol treated diabetic group. The *P* - value is > 0.01, hence the result is statistically significant at 1% level between diabetic control and ethanol treated diabetic group.

4. CONCLUSION

Antioxidant enzymes show a beneficial role in oxidative stress. The antioxidant SOD may contribute to the reduction of lipid peroxidation product MDA in ethanol treated diabetic rats. From this study it shows that a minute percentage of daily alcohol consumption does not affect the blood glucose and antioxidant level in diabetic and non-diabetic rats. Further detailed study will be required to establish the pharmacological approaches of alcohol towards diabetic therapy.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws were applicable. All experiments have been examined and approved by the Institutional Animal Ethics Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dominguez C, Ruiz E, Gussinye M, Carrascasa A. Oxidative stress at onset and in early stages of type 1 diabetes in children and adolescents. Diabetes Care. 1998;21(10):1736-1742.
2. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5:9-19.
3. Haldar PK, Kar B, Bhattacharya S, Bala A, Kumar RBS. Antidiabetic activity and modulation of antioxidant status by sansevieria *Roxburghiana rhizome* in streptozotocin-induced diabetic rats. Diabetol Croat. 2010;39(4):115-123.
4. Cahill A, Cunningham CC, Adachi M, Ishii H, Bailey SM, Fromenty B, Davies A. Effects of alcohol and oxidative stress on liver pathology: The role of the mitochondrion. Alcohol: Clin Exp Res. 2002;26(6):907-915.
5. Polavarapu R, Spitz DR, Sim JE, Follansbee MH, Oberley LW, Rehemtulla A, Nanji AA. Increased lipid peroxidation and impaired antioxidant enzyme function is associated with pathological liver injury in experimental alcoholic liver disease in rats fed diets high in corn oil and fish oil. Hepatology. 1998;27(5):1317-1323.
6. Bosch MF, Martnez S, Collel A. Chronical ethanol feeding induces cellular antioxidants decrease and oxidative stress in rats peripheral nerves. Free Radical in Biol Sci Med. 1998;25:365-368.
7. Available:www.who.int/diabetes/facts 2013.
8. Update 2014. International Diabetes Federation (IDF). Retrieved 29 November 2014.
9. Wannamethee SG, Shaper AG, Perry IJ, Alberti KGMM. Alcohol consumption and the incidence of type II diabetes. J Epidemiol Community Health. 2002;56: 542-548.
10. Ajani UA, Hennekens CH, Spelsberg A. Alcohol consumption and risk of type 2 diabetes mellitus among US male physicians. Arch Intern Med. 2000;160: 1025-1030.
11. Castelnuovo ADi, Costanzo S, Donati MB, Iacoviello L, Gaetano Gde. Prevention of cardiovascular risk by moderate alcohol consumption: Epidemiologic evidence and plausible mechanisms. Intern Emerg Med. 2010;5:291-297.
12. Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos Univ Med J. 2012;12(1):5-18.
13. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahu A, Bora U. Indian

medicinal herbs as sources of antioxidants. Food Res Int. 2008;41:1-15.
14. Malini P, Kanchana G, Rajadurai M. Antiperoxidative and antioxidant effect of ellagic acid on normal and streptozotocin induced diabetes in albino wistar rats. Res Pharm Biol Chem Sci. 2011;2(3):24-34.
15. Niedowics DM, Daleke DL. The role of oxidative stress in diabetic complications. Cell Biochem Biophys. 2005;43:289-330.
16. Neelakandhan SV, Rajasekaran N, Kanniappan GV, Devaki K. *In-vivo* antioxidant activity of Premna corymbosa (R ottl.) against streptozotocin induced oxidative stress in Wistar albino rats. Journal of Applied Pharmaceutical Science. 2012;2(10):060-065.
17. Bindu MP, Annamalai PT. Combined effect of alcohol and cigarette smoke on lipid peroxidation and antioxidant status in rats. Indian J Biochem Biophys. 2004;41:40-44.
18. Das SK, Vasudevan DM. Effect of ethanol on liver antioxidant defense systems: A dose dependent study. Indian J Clin Biochem. 2005;20(1):80-84.
19. Desco MC, Asensi M, Marquez R, Valls JM, Vento M, Pallardo FV , Sastre J, Vina J. Xanthine oxidase is involved in free radical production in type I diabetes protection by allopurinol. Diabetes. 2002; 51:1118-1124.
20. Sultatos LG. Effects of acute ethanol administration on the hepatic xanthine dehydrogenase / xanthine oxidase system in the rat. J Pharmacol Exp Ther. 1988; 246:946-949.
21. Kakkar R, Mantha SV, Radhi J, Prasad K, Kalra J. Increased oxidative stress in rat liver and pancreas during progression of streptozotocin - induced diabetes. Clin Sci. 1998;94:623-632.
22. Yoshikawa T, Naito Y. What is Oxidative Stress. Japan Med Assoc J. 2002;45(7): 271-276.
23. Kono Y. Generation of superoxide radical during auto-oxidation of hydroxylamine and assay for superoxide dismutase. Arch Biochem Biophys. 1978;186:189-195.
24. Noori S, Rehman N, Qureshi M and Mahboob T. Reduction of carbon tetrachloride-induced rat liver by coffee and green tea. Pakistan Journal of Nutrition. 2009;8(4):452-458.
25. Wills ED. Mechanisms of lipid peroxide formation in animal tissues. Biochem J. 1966;99:667-676.
26. Vinay K, Melissa L, Mansi S, Varsha S. Antioxidant and DNA damage protecting activities of *Eulophia nuda* Lindl. Free Radicals and Antioxidants. 2013;3:55-60.
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem; 1951.
28. Yin P, Zhao S, Chen S, Liu J, Shi L, Wang X, Liu Y, Ma C. Hypoglycemic and Hypolipidemic Effects of Polyphenols from Burs of *Castanea mollissima* Blume. Molecules. 2011;16:9764-9774.
29. Ogugua VN, Aroh AC. Effects of alcohol on oxidative parameters of alloxan induced Diabetic albino rat. Animal Research International. 2006;3(3):570-572.
30. Shanmugam KR, Mallikarjuna K, Reddy KS. Effect of alcohol on blood glucose and antioxidant enzymes in the liver and kidney of diabetic rats. Indian J Pharmacol. 2011; 43(3):330-335.
31. Issabeagloo E, Forughi R, Taghizadieh M, Kermanizadeh P. Effect of alcohol on blood glucose levels in streptozotocin induced rats. Middle - East J Sci Res. 2012;12(3): 290-293.
32. Van de Wiel A. Diabetes mellitus and alcohol. Diabetes Metab Res Rev. 2004;20(4):263-7.
33. Raman BV, Krishna ANV, Prakash VC, Saradhi MP, Samuel LA, Ramani GU, Varadacharyulu NC. Ameliorative effects of alcohol on human diabetic volunteers - a prospective study. Br J Pharm Res. 2014; 4(9):1067-1078.
34. Olayinka ET, Ore A, Adeyemo OA. Alterations in Biochemical indices and antioxidant status in rats following treatment with gatifloxacin. Br J Pharm Res. 2015;6(5):293-305.
35. Husain K, Scott BR, Reddy SK, Somani SM. Chronic ethanol and nicotine interaction on rat tissue antioxidant defense system. Alcohol. 2001:25(2): 89-97.
36. Dinu D, Nechifor MT, Movileanu L. Ethanol-induced alterations of the antioxidant defense system in rat kidney. J Biochem Mol Toxicol. 2005;19(6):386-395.
37. Das SK, Nayak P, Vasudevan DM. Biochemical markers for alcohol consumption. Indian J Clin Biochem. 2003;18(2):111-118.
38. Ramesh B, Karuna R, Sreenivasa RS, Haritha K, Sai MD, Sasi BR, Saralakumari D. Asian Pac J Trop Biomed. 2012;2(11): 895-900.

39. Pavana P, Sethupathy S, Manoharan S. Antihyperglycemic and antilipidperoxidative effects of *Tephrosia purpurea* seed extract in streptozotocin induced diabetic rats. Indian J Clin Biochem. 2007;22(1):77-83.
40. Feillet CC, Rock E, Coudray C, Grzelkowska K, Azais BV, Dardevet D, Mazur A. Lipid peroxidation and antioxidant status in experimental diabetes. Clin Chim Acta. 1999;284:31-43.
41. Noeman SA, Hamooda HE, Baalash AA. Biochemical study of oxidative stress Markers in the liver, kidney and heart of high fat diet induced obesity in rats. Diabetol Metab Syndr. 2011;3:17.

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