AMELIORATIVE EFFECTS OF ALCOHOL ON HUMAN DIABETIC VOLUNTEERS – A PROSPECTIVE STUDY

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ABSTRACT

Aims: The purpose of this study is to assess and confirm the ameliorative effects of alcohol consumption on biochemical indices of blood i.e., blood glucose, HbA1c, NO_2, NO_3, lipid profiles, hs-CRP (high sensitive C–Reactive protein) and membrane lipid peroxidation of diabetics.

Study Design: Pre-clinical and Biochemical experimental study.

Place and Duration of Study: Department of Biochemistry, Acharya Nagarjuna University & Dept. of Biotechnology, K L University, Guntur, A.P and Dept. of Biochemistry, Katuri Medical College, Katuri Nagar, Guntur, A.P and Dept. of Biochemistry, Sri Krishna Devaraya University, Anantapur, A.P and Dept. of Basic Sciences, Madanapalle Institute of Technology & Science (MITS), Post Box No: 14, Angallu (V), Madanapalle, A. P., India, during 2008 – 2013.

Methodology: The study is conducted on 4 groups (n= 1200) of people of different ages ranging from 35 to 50 years at community health centers in Prakasam, Warangal, Srikakulam districts of Andhra Pradesh, India. The first group consists of type-II diabetic patients who have been consuming alcohol (arithmetic mean ranging from 14.16 to 31.61ml/day) moderately for the past 3 to 10 years. The second group consists of non-diabetic, moderately alcohol consuming healthy individuals. The third group consists of patients who are type-II diabetics (who do not drink) taking medical treatment for minimum period of 1 year. The fourth group consists of non-drinking, non-diabetic healthy individuals. Relationships of alcohol intake with lipid profile, hs-CRP and HBA1c are compared among the three groups.

Results: In lipid profile analysis of moderately drinking diabetic group, the HDL levels are found to be higher while the remaining factors such as total cholesterol, LDL, VLDL ($P<0.05$), triglycerides ($P<0.01$) and membrane lipid peroxidation are significantly lower. Fasting serum glucose levels are lowered, while serum nitrites and nitrates are found to be significantly higher. These differences are not found in abstainers group and Diabetic group who do not drink.

Conclusion: Moderate consumption of alcohol in diabetic individuals is found to have an inverse association with the risky factors like LDL cholesterol, Triglycerides, etc. that are the etiological factors for some of the sequelae of diabetes mellitus i.e., coronary heart diseases, Retinopathy, etc. and has a direct association with the positive factors such as HDL and nitric oxide production. Experimental results are very significant and indicate that moderate consumption of alcohol has ameliorative effects on diabetics.

Keywords: diabetics, moderate drinkers, lipid profiles, Nitrites & Nitrates, HDL and HbA1c.

1. INTRODUCTION

Diabetes is a disorder where the body does not produce insulin or does not properly use insulin. According to recent estimates, approximately 285 million people worldwide (6.6%) are suffering from diabetes and this number is expected to rise by 438 million people (7.8%) of the adult population by 2030 [1, 2, 3, 4]. Glucose is derived from all sorts of foods that we consume. After every meal a large part of our food is converted into glucose, thereby increasing the blood glucose levels. The Insulin, a hormone secreted by pancreas carries the blood glucose to cells that need energy [5, 6]. In diabetic individuals, insulin is either not produced or not utilized properly, and hence the glucose remains in the blood causing the condition “Diabetes” [7, 8]. Today, diabetes mellitus type 2 is posing several challenges to the medical field due to its association with multiple physiological complications such as Cardiovascular complications, Microangiopathy, Neuropathy, Nephropathy, Retinopathy, Dermatopathy, etc [9]. Currently

oral hypoglycaemic agents used for the treatment of Type 2 diabetes include insulin secretagogues like sulfonylureas and metformin. Metformin acts through multiple poorly characterized mechanisms, one of which inhibits de novo glucose synthesis via indirect AMP-activated protein kinase (AMPK) activation, potentially following partial mitochondrial complex I inhibition in the liver [10]. Recently, the focus has been shifted towards the use of moderate alcohol to treat Type 2 diabetes. Alcohol consumption is increasing day by day, not only in Asian countries but also throughout the world. Alcohol is a globally abused psycho-active drug with its adverse side effects but it has also some important beneficial effects like relaxation of mental tension, vasodilatory effect on human health [11]. Excessive consumption of alcohol has definite adverse effect on human health. Several studies have shown that people with the habit of excessive drinking of ethanol are found to have fatty liver [12], cognitive disorders and permanent irreversible liver damage. On the other hand, it is also shown that moderate consumption of alcohol has beneficial health effects [13, 14, 15]. The concept of moderate consumption of ethanol (beverage alcohol) has evolved over time from considering the level of intake to be non-intoxicating and non-injurious. Moderate drinking can be defined as the level corresponding to the lowest overall rate of morbidity or mortality in a population [16].

Therefore, in our study we have evaluated the ameliorative effects of alcohol consumption on biochemical indices on human diabetic volunteers. The main criteria to study on these groups is the 'diabetes' a debilitating multifactorial disorder, is keep on increasing in Southern parts of India from the past two decades that coinside with intake of alcohol from last twenty years (*unpublished data*). We wanted to study the relationship between alcohol intake with diabetes and their ameliorative effects among the groups in these particular regions of Andhra Pradesh. Our results show that moderate alcohol consumption enhanced the levels of HDL by lowering LDL and total triglycerides pools. Moreover, enhanced levels of serum NO_2 and NO_3 are noticed in moderate alcohol drinking diabetic as well as non-diabetic volunteers.

2. MATERIALS AND METHODS

2.1 Subjects for study

The study is conducted on 4 groups of people of different ages ranging from 35 to 50 years at community health centers in Prakasam, Warangal, Srikakulam Districts of Andhra Pradesh, India. The first group consists of type-II diabetic patients who have been consuming alcohol moderately for the past 3 to 10 years. This group is named as MDD (Moderate Drinking Diabetics). The second group consists of non-diabetic moderately drinking healthy individuals called MDND (Moderate drinking non-diabetes). The third group consists of non-drinking type-II diabetic patients who have been under medical treatment for a minimum period of 1 year. This group is named as NDD (Non-Drinking Diabetics). The fourth group consists of non-drinking, non-diabetic healthy individuals. This group is Abstainers (Table 1). All

volunteers involved in the present study are well informed and their consent is obtained and size of the each group is 1200 keeping a total of 4800 individuals. All the members of the above groups are free from Coronary Heart Diseases (CHD), Cerebro Vascular Diseases (CVD) and Cancer.

2.2 Determination of fasting blood glucose

Blood samples from every individual are collected into EDTA containing tubes by venipuncture. Levels of glucose in serum are estimated using monozyme diagnostic kit, which is based on the GOD-POD method [17]. In brief, glucose is oxidized by the enzyme glucose oxidase to give D-gluconic acid and hydrogen peroxide. Hydrogen peroxide in presence of enzyme peroxidase oxidizes phenol, which combines with amino antipyrine dye to produce a red coloured quinoneimine which is measured at 505 nm against water blank.

2.3 Determination of serum triglycerides

Serum triglycerides are estimated using Qualigens diagnostic kit which is based on the method [18]. In brief, triglycerides in the sample are hydrolyzed by microbial lipase to glycerol and free fatty acids. Glycerol is further phosphorylated to glycerol 3-phosphate and is oxidized to dihydroxy acetone phosphate. Liberated hydrogen peroxide reacts with 4-amino anti pyrine and 3, 5 dichloro 2-hydroxy benzene sulphonic acid. Absorbance of quinoneimine and colour dye formed is proportional to the concentration of triglycerides.

2.4 Determination of Serum Total Cholesterol

Serum total cholesterol is estimated by the enzymatic kit method [19]. In brief 0.01ml of serum is added to 1ml of freshly reconstituted enzyme reagent, mixed well and incubated at 37^0C for 5 minutes. After incubation, absorbance is measured at 505nm against blank. Simultaneously standards are run along with the test under similar conditions.

2.5 Determination of HDL and LDL -Cholesterol

Serum HDL-Cholesterol is estimated by autozyme diagnostic kit method. 0.5ml of HDL precipitant reagent (Phosphotungstic acid 2.4 mmol/L and Magnesium Chloride 40m mol/L) is added to 0.5ml of serum, mixed thoroughly, centrifuged at 4,000 rpm for 10min to obtain a clear supernatant. 1ml of working standard (enzymatic cholesterol reagent of autozyme diagnostic kit) is added to 0.05ml of supernatant, incubated for 10min at 37^0C and the development of color is read at 510 nm against a blank. A standard is maintained simultaneously. LDL and VLDL cholesterol are calculated using the formula of [20].

2.6 Determination of CRP protein in serum

Cholestech LDX hs-CRP is an *in vitro* diagnostic test for the quantitative determination of hs – CRP (high sensitive C–Reactive protein) in whole blood or serum [26]. Finger stick samples are collected using a Cholestech LDX 50 µl capillary tube. The cassette is placed into the drawer of the analyzer immediately after dispensing the sample into the well. After pressing run, hs-CRP results are displayed in 6 minutes

(results are displayed in 4 minutes for serum of serum sample). It is found that Hematocrit levels between 30% and 55% do not affect the results.

2.7 Determination of total blood Nitrite and Nitrate

Nitrites and Nitrates are estimated in the serum samples of the subjects [22, 23]. Serum samples are deproteinated by adding 30% $ZnSO_4$ followed by centrifugation at 10,000 rpm for 5 minutes. Then, 1ml of serum supernatant is mixed with 1ml Greiss reagent (1g/lit sulfanilamide, 25g/lit phosphoric acid and 0.1gm/lit N-(1-Naphthyl) ethylene diamine dihydro chloride) and incubated at room temperature for 10 minutes for color development. The absorbance is measured at 545 nm in Elico Spectrophotometer against blank.

2.8 Statistical Analysis

All the values of body weight, fasting blood sugar, and biochemical estimations are expressed as mean ± standard deviation (S.D). Differences of mean values are assessed by using large sample Normal test z follows N (0, 1) because the sample size is 1200 (>30).

Methodology: Null hypothesis (H_0) → There are no significant difference between MDD and NDD, MDND and Abstainers.

Alternative hypothesis (H_1): Moderate drinking alcohol is beneficial in both the cases Diabetic as well as non-diabetic (One tailed test)

Under null hypotheses the test statistic is given by

$$Z = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \sim N(0,1)$$

If calculated value of Z ≤ Z_α (Critical value) we accept H_0 at $\alpha\%$ level of significance, otherwise we reject H_0 and conclusions can be drawn accordingly.

3. RESULTS AND DISCUSSION

Diabetes is a complex metabolic disorder and several factors such as environmental and life style factors have shown to be responsible for the origin and development of diabetes mellitus. Although diabetes is as old as human life on earth, researchers are yet to find out a therapeutic factor with less diabetic complications. In this paper, the authors explore the possible action of alcohol intake and diabetic control by measuring several biochemical indices in the blood serum of diabetics. However, the alcohol content in different drinks i.e., wine, brandy, whisky and other beverages varies considerably [24]. Therefore, a questionnaire has been prepared to know the type of drink consumed by MDD and MDND, which are shown in table 2. Based on that we calculated the arithmetic mean consumption of ethanol per day drunk by MDD and MDND, which ranges from 14.16 ml to 31.85 ml. Evaluation of the blood samples shows that moderate consumption of alcohol positively influences the indices of blood parameters of diabetics as well as non-diabetics i.e., hs-CRP protein, fasting blood glucose, HbA1c, total blood Nitrite and Nitrate,

total cholesterol, HDL, LDL, VLDL, Triglycerides and membrane lipid peroxidation and hence it is useful to ameliorate the deleterious effects of diabetes mellitus.

People who have habit of drinking alcohol (either heavy or moderate) have their own choice of drinking their selected brand. However, the alcohol content in different drinks i.e., wine, brandy, whisky and other beverages varies considerably [24]. Therefore, it is worthwhile to know the impact of alcohol on mucosal surface hence lipid peroxidation has great importance. In our experiments results showed that membrane lipid peroxidation is greatly lowered in MDD than NDD. In MDND the lipid peroxidation is higher than Abstainers due to the impact of alcohol on membrane. This data clearly shows that the ameliorative action of moderate alcohol on diabetic individuals than non-diabetic individuals. Similar results are also observed in hs-CRP and HbA1c levels (Table 3). In lipid profile analysis, only HDL levels are increased in MDD and MDND than NDD while remaining factors such as total cholesterol, LDL, VLDL ($P<0.05$), membrane lipid peroxidation and triglycerides ($P<0.01$) are significantly reduced (Table 4). Both the study groups (MDD & NDD) are compared with the MDND and abstainers. These results on lipid profile due to the impact of alcohol consumption are supported by several authors who conducted experiments on different animals including human beings [25]. Similar experiments are conducted on men with and without diabetes and a positive association between alcohol intake and blood pressure, triglycerides and HDL cholesterol is found [26]. Some researchers may still have a doubt whether excessive consumption of alcohol may result in obesity. But, this ambiguity is already resolved by [27] who observed that drinkers, despite their higher alcohol intake, are no more obese than nondrinkers. Their observations strongly complement the explanation of present study especially referring the mean BMI in MDD and MDND (Table 1). Several reports are already noticed that driking of alcohol once or twice a week will reduce the body weight while every day driking gain more weight. This is due to the stimulatory effect of alcohol on metabolism. In our experiments it is noticed that BMI is less in MDD compared with NDD because of moderate drinking. While comparing MDD and MDND, surprisingly BMI is higher in MDD than MDND; this is due to the impact of diabetes on body. In MDND, moderate alcohol comsumption gives very significant lowering impact on BMI. However, our earlier reports revealed that those who are drinking either moderately or heavily have an habit of eating too much fatty foods during the drinking in most of the cases, causes display of higher BMI in all such individuals (unpublished data). Therefore, we clearly seen this fact when campared the BMI of MDND with Abstainers.

The levels of serum Nitrites and Nitrates are found to be increased in MDD and MDND as compared to NDD ($P<0.05$) (table 3). Earlier studies revealed that moderate alcohol consumption might have induced an increase in insulin secretion, sensitivity to insulin, increased serum nitrites and nitrates levels in MDD and MDND than NDD. Relationship between serum Nitric Oxide (NO) production, lipid abnormalities and oxidative stress in diabetes are noticed earlier [28]. Many reports strongly support that diabetes mellitus is associated with decreased Nitric Oxide production from endothelial cells and decreased levels of serum NO_2 and NO_3 [28, 29, 30, 31]**.** Moderate alcohol consumption has been shown to reduce the risk of

ischemic heart disease potentially through its effect on specific endothelial-derived compounds. Venkov et al., [32] have tested the hypothesis that ethanol increases the expression of endothelial Nitric Oxide Synthase (eNOS) and Nitric Oxide production in Bovine aortic endothelial cells. Luo et al., [33] and Bequette et al., [34] observed that intake of alcohol has direct influence on wound healing and ascribed this property of alcohol to increased production of NO which, as a vasodilator, helps in healing the wound. In fact, alcohol rubbed on skin dilates the blood vessels and produces a mild counter-irritant effect. In the general practice of public, whenever a small cut/injury appears on the body, people pour a few drops of alcohol on the injured part and the wound gets healed subsequently. Other reports also strongly suggest that increased production of nitric oxide in alcoholic diabetics reduces the serum glucose levels, oxidative stress, lipid and lipoprotein abnormalities [18, 35, 36, 37, 38, 39]. The etiological factor for most of the sequelae of diabetes mellitus of type I or II i.e., Retinopathy, Nephropathy, Cardio-myopathy, Polyneuropathy, Neuritis, Erectile and Dysfunction is ischemia due to lowered levels of Nitric Oxide production. Hence, the authors opine that moderate consumption of alcohol ameliorates the severity of diabetes mellitus and its sequelae to some extent due to increased nitric oxide synthase protein expression of one or more isoforms.

The moderate consumption of alcohol causes a significantly alleviated in serum glucose ($P<0.05$) and glycosylated hemoglobin levels in MDD than NDD and MDND than Abstainers as observed earlier through similar experiments conducted on moderately drinking type-II diabetics [40, 41]**.** These effects are purely ameliorated by moderate alcohol consumption. In contrast to the fasting serum gluceose levels, the serum Nitrite and Nitrate levels are improved in MDD than NDD and in MDND than Abstainers. According to the Venkov et al., it is revealed that the moderate alcohol consumption enhance the endothelial Nitric Oxide Synthase (eNOS) and Nitric oxide production (33 & 34). This data is in support to our results and prove the direct impact of moderate alcohol on diabetic individuals. Similar results are reported by [42] conducting experiments on rats where they demonstrated that ethanol acutely exerts substantial influences on pancreatic microcirculation by evoking a massive redistribution of pancreatic blood flow from the exocrine into the endocrine part via mechanisms mediated by nitric oxide and vagal stimuli, augmenting late-phase insulin secretion, and thereby evoking hypoglycemia. This mechanism seems to involve NO & vagal pathways and is due to the well-known hypoglycemic properties of alcohol in diabetic patients [43, 44]. A Dutch randomized trial conducted in diabetic teetotallers suggests that a glass of wine with dinner may improve glucose control, particularly in those with higher HbA1c levels to begin with. This study, while small, adds to anecdotal evidence and meta-analyses that suggest that wine may hold specific benefits for diabetics whose cardiovascular benefits have been widely touted (European Association for the study of Diabetes 2007 meeting, an unpublished report). Experimental studies on the composition of alcohol stating that the principal ameliorative effect of the alcohol on diabetics is due to the presence of polyphenols as ingredients (45). However, it is evident that levels of polyphenolic metabolites that reach the human body are always very low (46). Therefore, it is clear that

the moderate alcohol consumption along with polyphenols have involved in the alleviation of glucose levels in MDD and MDND.

Consumption of white and red wines may improve coronary blood flow and improve symptoms in patients with coronary heart diseases [47]. In our experiments, it is observed that hs–CRP levels in blood serum are found to be significantly ($P<0.05$) low in MDD and MDND when compared with that of NDD, which indicates that the probable risk of cardiovascular diseases is low in MDD (Table 3).

Glycosylated hemoglobin (Hemoglobin A1c) concentration is a hallmark of glycemic control for prognostic purpose. HbA1c levels are reported to be in correlation with, not only glycosuria but also serum glucose. Hormonal profiles and various other factors cannot influence HbA1c concentrations [34]. Our experiments on HbAlc levels in the MDD, MDND and NDD patients show that lowered levels of blood glucose exist in MDD and MDND than NDD. These results strongly support our hypothesis that moderate consumption of alcohol has an ameliorative effect on diabetes mellitus. As the results are very significant, the authors propose that moderate consumption of alcohol (ranging from 14.16 ml to 31.85 ml per day) is good for the health of the diabetics. This range is very much below the safer range i.e., 30 to 40 ml of ethanol consumption/day as advised by the UK government (International center for Alcohol Policies, USA).

4. CONCLUSION:

Moderate consumption of alcohol in diabetic individuals is found to have an inverse association with the risky factors like LDL cholesterol, Triglycerides, etc. that are the etiological factors for some of the sequelae of diabetes mellitus i.e., coronary heart diseases, Retinopathy, etc. and has a direct association with the positive factors such as HDL and nitric oxide production. Experimental results are very significant and indicate that moderate consumption of alcohol has ameliorative effects on diabetics.

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COMPETING INTERESTS

None declared

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TABLES & LEGENDS

Table -1: Profile of subject groups with and without diabetes

Variables	Alcohol consumption category			
	Moderate drinking Diabetes (MDD)	Non-drinking Diabetes (NDD)	Moderate Drinking non-Diabetes (MDND)	Abstainers
Gender	Male	Male	Male	Male
Number	1200	1200	1200	1200
Age (Years)	52.2 ± 7.8	52.2 ± 7.8	52.2 ± 7.8	52.2 ± 7.8
Body mass index (Kg/m^2)	25.28 ± 3.27*	28.32 ± 5.21	25.61 ± 0.06†	23.32 ± 2.39
Waist Circumference (Inches)	85.9 ± 13.2*	89.4 ± 15.2	89.3 ± 8.2†	84.3 ± 10.2
Daily consumption of moderate alcohol (≥22 and ≤44 g ethanol/day)	All*	Nil	All†	Nil
Smokers (%)	52.4	42.5	60.5	Nil
Systolic blood pressure (mmHg)	132.4 ± 17.2*	138.4 ± 17.8	132.65 ± 2.7†	130.3 ± 16.7
Diastolic blood pressure (mmHg)	81.4 ± 12.1*	82.4 ± 13.1	86.4 ± 5.4†	80.4 ± 11.1
Therapy for diabetes (%)	58.7*	59.3	Nil	Nil
Therapy for hypertension (%)	42.2*	35.8	15.8	8.0
Therapy for dyslipidemia (%)	15.9*	23.7	10.6	8.2

Mean with standard deviation or percentages of variables were compared between the non-diabetic and diabetic with drinkers and non-drinkers.

†Dose size is 13.0 to 40.0 % ABV (Percent Alcohol by Volume from typical beverage.

* Significant variation observed between MDD and NDD (Calculated Z>1.645)

† Significant variation observed between MDND and Abstainers (Calculated Z>1.645)

Table - 2: Calculation of ethanol content in drinks consumed by MDD.

S. No.	Type of Drink	ABV* (%)	Daily consumption of drink ** (in ml)	Daily consumption of ethanol ***(in ml)
1	Wine	13.5	105.00	14.16
2	Brandy	40	77.65	26.76
3	Rum	37.5	80.00	30.00
4	Gin	40	71.25	28.50
5	Whisky	40	79.62	31.85
6	Cheap Liquor	40	79.02	31.61

* Typical Alcohol by Volume; ** Arithmetic mean alcohol consumption by MDD in a week time;
*** Arithmetic mean alcohol consumed equivalent to ethanol per week (i.e., 220 ml)

TABLE-3: Impact of alcohol on biochemical parameters of serum and erythrocyte membrane in different experimental groups

S. No.	Biochemical Parameter	Alcohol consumption category			
		Moderate drinking Diabetes (MDD)*	Non-drinking Diabetes (NDD)	Moderate Drinking non-Diabetes (MDND) **	Abstainers
1	Fasting serum glucose (mg / dl)	130 ± 4.3	180 ± 7.0	94 ± 2.2	72± 2.3
2	hs-CRP (mg/L)	2.54 ± 0.05	3.12 ± 0.03	2.37 ± 0.03	1.3± 0.06
3	HbA1c[†]	9.5 ± 2.3	11.4 ± 2.2	6.55 ± 0.05	6.5± 1.0
4	Serum Nitrites (µ moles/L)	2.5 ± 0.04	2.3 ± 0.03	2.33 ± 1.03	1.6± 1.0
5	Serum Nitrates (µ moles/L)	24.5 ± 0.4	22.7 ± 0.5	24.6 ± 6.7	23.1 ± 8.9
6	Membrane Lipid peroxidation *(pmol* of MDA) [††]	4.961 ± 1.15	8.304 ± 1.026	5.542 ± 1.026	3.20 ± 0.15

[†]*Determined using Glycated hemoglobin assay kit recommended by the American diabetes association (ADA) and is expressed as a percentage (%) of the hemoglobin*

[††]Malonaldehyde formed / mg membrane protein

***** Significant variation observed between MDD and NDD (Calculated Z>1.645)

******Significant variation observed between MDND and Abstainers (Calculated Z>1.645)

TABLE-4: Variation in the lipid profiles of diabetic volunteers with and without drinking

S. No.	Parameter (mg / dl)	Alcohol consumption category†			
		Moderate drinking diabetes (MDD)*	Non-drinking Diabetes group (NDD)	Moderate Drinking non-Diabetes (MDND)**	Abstainers
1	Total Cholesterol	220 ± 8.4	265 ± 7.8	188 ± 05	198 ± 8
2	Triglycerides	170 ± 8.5	250 ± 5.3	152 ± 07	142 ± 29
3	HDL	82 ± 5.1	53 ± 3.7	64 ± 3.3	42 ± 1.8
4	LDL	51 ± 3.6	59 ± 4.0	61 ± 2.8	60 ± 10
5	VLDL	35 ± 3.1	48 ± 3.6	32 ± 1.6	38 ± 2.0

†Values (n=1200) represented as mean values ± S.D.

* Significant variation observed between MDD and NDD (Calculated Z>1.645)

**Significant variation observed between MDND and Abstainers (Calculated Z>1.645)