

Ameliorative Effects of Alcohol on Human Diabetic Volunteers – A Prospective Study

ABSTRACT

Aim:The purpose of this study was to assess and confirm the ameliorative effects of alcohol consumption on biochemical indices of blood viz. blood glucose, HbA1c, NO₂, NO₃, lipid profiles, hs-CRP (high sensitive C–Reactive protein) and membrane lipid peroxidation of diabetics. **Methods:** The study was conducted on 3 groups of people of age ranging from 35 to 50 years at community health centers in Prakasam district, Warangal district, Srikakulam District of Andhra Pradesh, India. The first group consists of 298 male 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 110 patients who are type-II diabetics (who do not drink) taking medical treatment for minimum period of 1 year. The third group consists of 100 non-drinking, non-diabetic healthy individuals. Relationships of alcohol intake with lipid profile, hs-CRP and HBA1c were compared among the three groups. **Results:** In lipid profile analysis of moderately drinking diabetic group, the HDL levels were 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 were significantly lower. Fasting blood glucose, Plasma nitrites and nitrates were found to be significantly higher. These differences were not found in control group and Diabetic group who do not drink. **Conclusion:** Moderate consumption of alcohol 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 viz. coronary heart diseases, Retinopathy, etc. and has a direct association with the positive factors 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.

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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%) suffering with diabetes and 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 an 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 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 are being 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 etc., on human health (11). Excessive consumption of alcohol has definite adverse effect on human health. Several studies have shown that excessive drinking of ethanol are found to have fatty liver (12) cognitive disorders and permanent irreversible liver damage etc. On the other hand, it was also shown that moderate alcohol consumption has beneficial health effects (22, 33, 38). 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

60 can be defined as the level corresponding to the lowest overall rate of morbidity or mortality in a
61 population (30).

62 Therefore, in our study we have evaluated the ameliorative effects of alcohol consumption on biochemical
63 indices on human diabetic volunteers. Our results showed that moderate alcohol consumption enhanced
64 the levels of HDL by lowering LDL and total triglycerides pools. Moreover, enhanced levels of plasma NO₂
65 and NO₃ was noticed in moderate alcohol drinking diabetic volunteers.

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67 **2. MATERIALS AND METHODS**

68 **2.1 Subjects for study**

69 The study was conducted on 3 groups of people of age ranging from 35 to 50 years at community health
70 centers in Prakasam district, Warangal district, Srikakulam District of Andhra Pradesh, India. The first
71 group consists of 298 male type-II diabetic patients who have been consuming alcohol moderately for the
72 past 3 to 10 years. This group is named as MDDG (Moderate Drinking Diabetic Group). The second
73 group consists of 110 patients who are type-II diabetics (and they do not drink) taking medical treatment
74 for minimum period of 1 year. This group is named as DG (Diabetic group). The third group consists of
75 100 non-drinking, non-diabetic healthy individuals. This group is named as Control Group (CG). All
76 volunteers involved in the present study were well informed and their consent was obtained. All the
77 members of the above groups are free from Coronary Heart Diseases (CHD), Cerebro Vascular Diseases
78 (CVD) and Cancer.

79 **2.2 Statistical Analysis**

80 Differences of mean values were assessed by paired or unpaired Student's *t* test for comparison of 2
81 variables and by ANOVA for comparison of multiple variables. Relationships between 2 continuous
82 variables were assessed by a regression analysis using the Pearson correlation coefficient. Differences
83 between Alcoholic and Non-alcoholic diabetic groups were analyzed by χ^2 test. A value of $P < 0.05$ was
84 considered statistically significant.

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87 2.3 Determination of fasting blood glucose

88 Blood samples from every individual were collected into EDTA containing tubes by venipuncture. Levels
89 of glucose in plasma is estimated using monozyme diagnostic kit, which is based on the GOD-POD
90 method (28). In brief, Glucose is oxidized by the enzyme glucose oxidase to give D-gluconic acid and
91 hydrogen peroxide. Hydrogen peroxide in presence of enzyme peroxidase oxidizes phenol, which
92 combines with amino antipyrine dye to produce a red coloured quinoneimine which is measured at 505
93 nm against water blank.

94 2.4 Determination of plasma triglycerides

95 Plasma triglycerides were estimated using Qualigens diagnostic kit which is based on the method (19). In
96 brief, triglycerides in the sample is hydrolyzed by microbial lipase to glycerol and free fatty acids. Glycerol
97 is further phosphorylated to glycerol 3-phosphate and is oxidized to dihydroxy acetone phosphate.
98 Liberated hydrogen peroxide reacts with 4-amino anti pyrine and 3, 5 dichloro 2-hydroxy benzene
99 sulphonic acid. Absorbance of quinoneimine and colour dye formed is proportional to the concentration of
100 triglycerides.

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102 2.5 Determination of Plasma Total Cholesterol

103 Plasma total cholesterol was estimated by the enzymatic kit method (13). In brief 0.01ml of plasma is
104 added to 1ml of freshly reconstituted enzyme reagent, mixed well and incubated at 37⁰C for 5 minutes.
105 After incubation, absorbance was measured at 505nm against blank. Simultaneously standards were run
106 along with the test under similar conditions.

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108 2.6 Determination of HDL and LDL -Cholesterol

109 Plasma HDL-Cholesterol was estimated by autozyme diagnostic kit method. 0.5ml of HDL precipitant
110 reagent (Phosphotungstic acid 2.4 mmol/L and Magnesium Chloride 40m mol/L) was added to 0.5ml of
111 plasma, mixed thoroughly, centrifuged at 4,000 rpm for 10min to obtain a clear supernatant. 1ml of
112 working standard (enzymatic cholesterol reagent of autozyme diagnostic kit) was added to 0.05ml of
113 supernatant, incubated for 10min at 37⁰C and the development of color was read at 510 nm against a

114 blank and a standard was run simultaneously. LDL and VLDL cholesterol were calculated using the
115 formula of (20).

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117 **2.7 Determination of CRP protein in serum**

118 Cholestech LDX hs-CRP is an *in vitro* diagnostic test for the quantitative determination of hs – CRP (high
119 sensitive C–Reactive protein) in whole blood or serum (26). Finger stick samples are collected using a
120 Cholestech LDX 50 µl capillary tube. Place the cassette into the drawer of the analyzer immediately after
121 dispensing the sample into the well. After pressing run, hs-CRP results will be displayed in 6 minutes
122 (results will be displayed in 4 minutes for serum or plasma sample). Hematocrit levels between 30% and
123 55% do not affect results.

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125 **2.8 Determination of total blood nitrite and nitrate**

126 Nitrites and Nitrates were estimated in the serum samples of the subjects (35, 37). Plasma samples were
127 deproteinated by adding 30% ZnSO₄ followed by centrifugation at 10,000 rpm for 5 minutes. Then, 1ml of
128 plasma supernatant was mixed with 1ml Greiss reagent (1g/lit sulfanilamide, 25g/lit phosphoric acid and
129 0.1gm/lit N-(1-Naphthyl) ethylene diamine dihydro chloride) and incubated at room temperature for 10
130 minutes for color development. The absorbance was measured at 545 nm in Elico Spectrophotometer
131 against blank.

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133 **3. RESULTS AND DISCUSSION**

134 Diabetes is a complex metabolic disorder and several factors such as environmental and life style factors
135 has shown to be responsible for the origin and development of diabetes mellitus. Although Diabetes is as
136 old as human life on earth, researchers are till to find out a therapeutic factor with less diabetic
137 complications. In this paper we explore the possible action of alcohol intake and diabetic control by
138 measuring several biochemical indices in the blood serum of diabetics. However, the alcohol content in
139 different drinks viz. Wine, Brandy, Whisky, etc. varies considerably (15). Therefore, we have prepared a
140 questionnaire form to know the type of drink consumed by MDDG, which is shown in table – I, based
141 on that we calculated the arithmetic mean consumption of ethanol per day drunk by MDDG, which

142 ranges from 26.76 ml to 31.85 ml. Evaluation of the blood samples showed that moderate consumption of
143 alcohol positively influences the indices of blood parameters of diabetics i.e., hs-CRP protein, fasting
144 blood glucose, HbA1c, total blood Nitrite and Nitrate, total cholesterol, HDL, LDL, VLDL, Triglycerides and
145 membrane lipid peroxidation and hence it is useful to ameliorate the deleterious effects of diabetes
146 mellitus.

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148 We observed from our results that membrane lipid peroxidation is declined in moderately drinking diabetic
149 group than diabetic and control groups (Table – II). In lipid profile analysis, only HDL levels were
150 increased in MDDG than DG while remaining factors such as total cholesterol, LDL, VLDL ($P<0.05$),
151 membrane lipid peroxidation and triglycerides ($P<0.01$) were significantly reduced (Table–III). Both study
152 groups were compared with control group. These results on lipid profile due to the impact of alcohol
153 consumption are supported by several authors who conducted experiments on different animals including
154 human (18). Similar experiments were conducted by on men with and without diabetes and they found
155 positive association between alcohol intake and blood pressure, triglycerides and HDL cholesterol in men
156 who consumed alcohol (42). Some researchers may still have a doubt whether excessive consumption of
157 alcohol may result in obesity. But, this ambiguity was resolved by (21) who observed that drinkers,
158 despite their higher alcohol intake, were no more obese than nondrinkers. Their observations strongly
159 complement our observations.

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161 The levels of plasma nitrites and nitrates are found to be increased in MDDG as compared to DG. Earlier
162 studies revealed that moderate alcohol consumption might have induced an increase in insulin secretion,
163 sensitivity to insulin, increased plasma nitrites and nitrates levels in MDDG than DG. Relationship
164 between plasma nitric oxide production, lipid abnormalities and oxidative stress in diabetes earlier noticed
165 (27). Many reports strongly support that diabetes mellitus is associated with decreased nitric oxide
166 production from endothelial cells and decreased levels of plasma NO_2 and NO_3 (27, 31, 34, 36). Moderate
167 alcohol consumption has been shown to reduce the risk of ischemic heart disease potentially through its
168 effect on specific endothelial-derived compounds (41) tested the hypothesis that ethanol increases the
169 expression of endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) production in Bovine aortic

170 endothelial cells (24, 43) observed that intake of alcohol has direct influence on wound healing and he
171 ascribed this property of alcohol to increased production of NO which, as a vasodilator, helps in healing of
172 the wound. As a general observation, it is found that alcohol rubbed on skin dilates the blood vessels and
173 produces a mild counter-irritant effect. In the general practice of public, whenever a small cut/injury
174 appears on the body, people pour a few drops of alcohol on the injured part and the wound gets healed
175 subsequently. Other reports also strongly suggest that increased production of nitric oxide in alcoholic
176 diabetics reduces the plasma glucose levels, oxidative stress, lipid and lipoprotein abnormalities (14, 16,
177 17, 39, 44, 19).

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179 In the present study, we found that moderate alcohol consumption enhanced the levels of plasma NO₂
180 and NO₃ in MDDG when compared to DG (P<0.05); this observation strongly coincides with above
181 reports. The etiological factor for the most of the sequelae of diabetes mellitus of type I or II viz.
182 Retinopathy, Nephropathy, cardio myopathy, polyneuropathy, neuritis, erectile dysfunction etc. , is
183 ischemia due to lowered levels of Nitric oxide production. Hence, the authors opine that moderate
184 consumption of alcohol ameliorates the severity of diabetes mellitus and its sequelae to some extent due
185 to increased nitric oxide synthase protein expression of one or more isoforms.

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187 The moderate consumption of alcohol causes a significant decrease in plasma glucose levels (P<0.05)
188 and glycosylated hemoglobin in MDDG than DG; as observed earlier through similar experiments
189 conducted on moderately drinking type-II diabetics (25, 45). Similar results were reported and (23)
190 conducting experiments on rats where they demonstrated that ethanol acutely exerts substantial
191 influences on pancreatic microcirculation by evoking a massive redistribution of pancreatic blood flow
192 from the exocrine into the endocrine part via mechanisms mediated by nitric oxide and vagal stimuli,
193 augmenting late-phase insulin secretion, and thereby evoke hypoglycemia. This mechanism seems to
194 involve NO & vagal pathways and is due to the well-known hypoglycemic properties of alcohol in diabetic
195 patients (32, 40). A Dutch randomized trial conducted in diabetic teetotallers suggests that a glass of wine
196 with dinner may improve glucose control, particularly in those with higher HbA1c levels to begin with. This
197 study, while small, adds to anecdotal evidence and meta-analyses that suggest that wine, whose

198 cardiovascular benefits have been widely touted, may hold specific benefits for diabetics (European
199 Association for the study of Diabetes 2007 meeting, an unpublished report).

200
201 Consumption of white and red wines may improved coronary blood flow and improve symptoms in
202 patients with coronary heart diseases (29). In our experiments, it was observed that hs-CRP levels in
203 blood serum are found to be significantly ($P<0.05$) low in MDDG when compared with that of DG, which
204 indicates that the probable risk of cardiovascular diseases is low in MDDG (Table – II).

205
206 Glycosylated hemoglobin (Hemoglobin A1c) concentration is a hallmark of glycemic control for prognostic
207 purpose. HbA1c levels are reported to be in correlation with, not only glycosuria but also serum glucose.
208 Hormonal profiles and various other factors cannot influence HbA1c concentrations (43). Our experiments
209 on HbA1c levels in the MDDG and DG patients show that lowered levels of blood glucose exist in MDDG
210 than DG. These results strongly support our hypothesis that moderate consumption of alcohol has an
211 ameliorative effect on diabetes mellitus. As the results are very significant, the authors propose that
212 moderate consumption of alcohol (ranging from 26. 76 ml to 31.85 ml per day) is good for the health of
213 the diabetics. This range is very much below the safer range i.e., 30 to 40 ml of ethanol consumption/day
214 as advised by the UK government (International center for Alcohol Policies, USA).

215 216 **ACKNOWLEDGMENTS**

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221 **COMPETING INTERESTS**

222
223 None declared

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357 **Tables**

358

359 **Table - I:** Calculation of ethanol content in drinks consumed by MDDG.

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S. No.	Type of Drink	ABV* (%)	Daily consumption of drink ** (in ml)	Content of ethanol in the drink*** (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

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* Alcohol By Volume (Typical); ** Arithmetic mean alcohol consumption of MDDG in a week equivalent to ethanol (i.e., 220 ml ethanol per week***)

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366 **Table-II:** Variation in the levels of different biochemical indices of moderately drinking diabetics; diabetics and control groups.

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S. No.	Parameter	Moderately Drinking Diabetic group (MDDG)	Diabetics group (DG)	Control group (CG)
1	Fasting Plasma Glucose*	130 ± 4.3	180 ± 7.0	72± 2.3
2	hs-CRP**	2.54 ± 0.05	3.12 ± 0.03	1.3± 0.06
3	Membrane Lipid peroxidation***	4.961 ± 1.15	8.304 ± 1.026	3.20 ± 0.15
4	HBA1c†	9.5 ± 2.3	11.4 ± 2.2	6.5± 1.0
5	Plasma Nitrites††	2.5 ± 0.04	3.3 ± 0.06	1.6± 1.0
6	Plasma Nitrates††	24.5 ± 0.4	33.7 ± 0.5	23.1 ± 8.9

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* mg / dl; **mg/L; *** pmol of MDA (Malonaldehyde) formed / mg membrane protein;

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†† μ moles/L; †Determined using Glycated hemoglobin assay kit recommended by the American diabetes association (ADA) and is expressed as a percentage (%) of the hemoglobin

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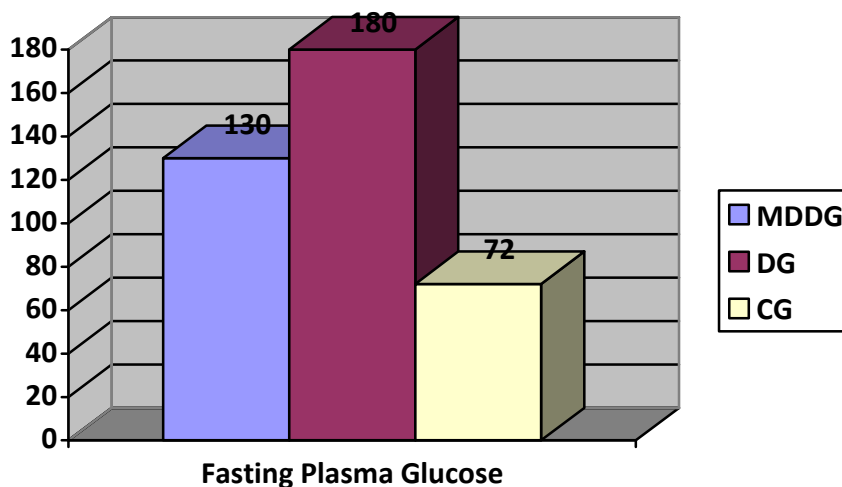
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Table-III: Variation in the lipid profiles of moderately drinking diabetics, diabetics & Control groups.

S. No.	Parameter (mg / dl)	Moderately Drinking Diabetic group (MDDG)	Diabetics group (DG)	Control group (CG)
1	Total Cholesterol	220 ± 8.4 ^a	265 ± 7.8 ^b	198±8 ^c
2	Triglycerides	170 ± 8.5 ^a	250 ± 5.3 ^b	142±29 ^c
3	HDL	82 ± 5.1 ^a	53 ± 3.7 ^b	42±1.8 ^c
4	LDL	51 ± 3.6 ^a	59 ± 4.0 ^b	60±10 ^c
5	VLDL	35 ± 3.1 ^a	48 ± 3.6 ^b	38±2.0 ^c

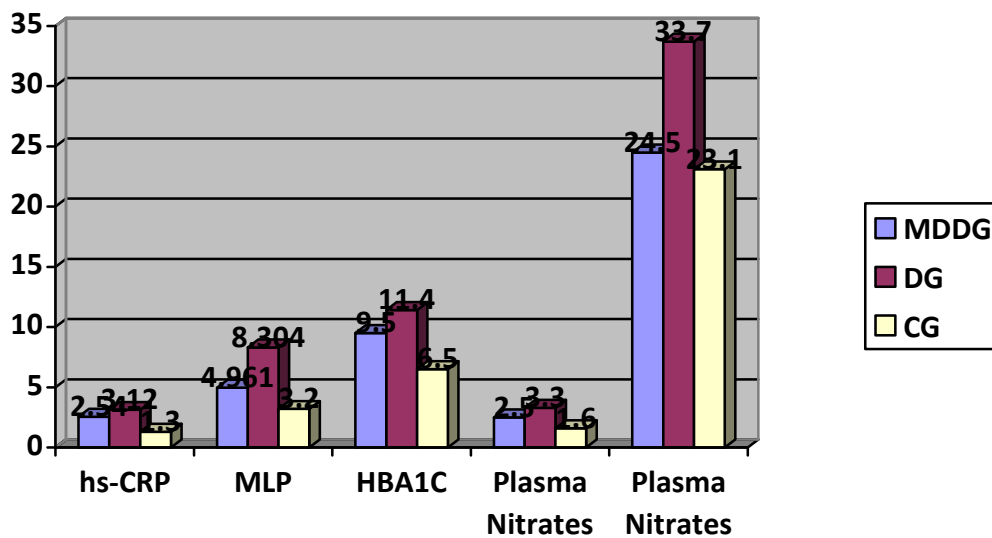
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^{a-c} Mean values (n=100) in each row followed by the same superscript letter.



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Graphical representation of the Fasting Plasma Glucose

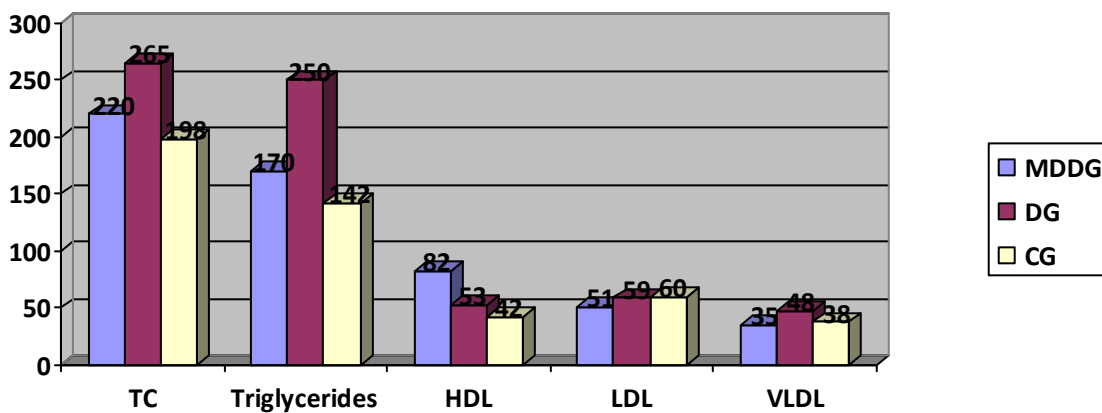


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Graphical representation of the Different Parameters

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Graphical representation of the Lipid Profile