Association of the types of alcoholic beverages and blood lipids in a local population in Jharkhand, India

Sunil Kumar Verma[1], Janmejaya Rout[2], Shrutidhara Biswas[3], Umakanta Tripathy[2*]

[1]Department of Biochemistry, Patliputra medical college, Dhanbad, Jharkhand, 826005, India

[2]Department of Applied Physics, Indian Institute of Technology (Indian School of Mines), Dhanbad, Jharkhand, 826004, India.

[3]Department of Biotechnology, Indian Institute of Technology, Guwahati, Assam, 781039, India.

[*]Corresponding author: utripathy@iitism.ac.in

Abstract: Although light-to-moderate alcohol consumption is considered beneficial, alcohol in binge doses or high cumulative lifetime consumption leads to cardiovascular diseases, metabolic syndrome and structural damage to various organs. Alcohol is known to alter blood lipid concentrations; however, the association of the types of alcohol on the lipid profile has not been investigated extensively. A cross-sectional study involving male participants (n = 86) aged 20 to 60 from the Ranchi and Dhanbad zone of Jharkhand, India, was carried out to investigate the effects of cumulative lifetime consumption of Haria, a local rice-based fermented alcohol, Indian made foreign liquor (IMFL), and a combination of the two on the blood lipid profiles. Demographic characteristics, dietary intake and medical history were obtained from the participants by questionnaire, and lipid levels were determined by analysis of blood samples. The effect of Haria alone on the blood lipids was also investigated on the local female population (n = 31). After adjusting for demographic and dietary factors, IMFL and combination of IMFL and Haria consumption was associated with increased serum total cholesterol, triglyceride, and low density lipoprotein (LDL) cholesterol levels ($P < 0.05$) and decreased high density lipoprotein (HDL) cholesterol levels ($P < 0.05$). None of the blood lipids changed significantly in Haria consumers in both male and female groups. This study suggests that Haria, a popular alcoholic beverage of West Bengal and east-central India, is a relatively safe local alcoholic beverage and does not alter the lipid profile in consumers.

Keywords: Alcohol, Cholesterol, Coronary artery disease, Haria, Lipid profile, Indian made foreign liquor, Lipoproteins.

Introduction

Over the last few decades, accumulating evidences suggest that light-to-moderate alcohol consumption may operate through an improved lipid profile [1] including increase in high density lipoprotein (HDL) cholesterol and apolipoprotein A-I, and, to a lesser extent, a decrease in low density lipoprotein (LDL) cholesterol [2-4] thus offering protection against cardiovascular diseases morbidity and mortality [5-7]. Although the definition of a moderate dose somewhat differs in the field, 2 drinks a day (20-30 g of alcohol) for men and one drink a day (10-15 g of alcohol) for women may be considered a moderate level of alcohol consumption [8].

On the other hand, high dose of alcohol consumption (> 60 g per day in men and > 40 g per day in women) [9] arising from binge doses or high cumulative lifetime consumption may induce a spectrum of cardiovascular diseases often accompanying arterial hypertension, diabetes and altered lipid profiles [10-16]. Owing to the global burden of diseases arising from excessive alcohol consumption, there are concerted efforts to promote moderation throughout the population, with interventions such as "Alcohol, less is better" [17, 18].

Interestingly, it is reported that heavy alcohol consumption, regardless of the type of alcoholic beverage consumed that includes beer, wine and spirits, result in significantly greater levels of HDL cholesterol, HDL3 cholesterol, and apolipoprotein A-I in both white and African-American males and females of the large Atherosclerosis Risk in Communities (ARIC) study [19]. In clear contrast to the earlier findings, this study reports that the alterations in the lipid profile of heavy drinkers appear similar to the light-to-moderate drinkers irrespective of the alcohol type [19]. It is therefore uncertain if different types of alcohol truly affect the lipid profile to a similar extent. It becomes imperative to investigate whether regular heavy drinkers in the Indian sub-population, where a significant percentage of drinking population consumes locally made alcohol, shows similar alteration in lipid profile in response to the consumption of different types of alcoholic beverage.

Here, we carried out a cross-sectional study to investigate the influence of different types of alcoholic beverages on blood lipid levels arising out of excessive alcohol consumption in the ethnic male population of Ranchi and Dhanbad zone of Jharkhand. Participants of this study were divided into four groups; non-drinking control group, Haria, a locally made rice-based fermented alcohol, drinking group, Indian made foreign liquor (IMFL) that includes whiskey, gin, rum, brandy and vodka consuming group and group that consumed both IMFL and Haria. The blood lipid profile that included serum total cholesterol, LDL cholesterol, serum triglyceride, and HDL cholesterol were assessed in these groups. We were surprised to find that Haria, one of the most popular local alcoholic beverages of West Bengal, Jharkhand and east-central India and heavily consumed in this region, showed no alteration in the lipid profiles not only in male but also in female drinking groups when compared to non-drinking gender matched controls. On the other hand, IMFL and combination of IMFL and Haria showed alterations in the lipid profile indicating detrimental effects of these on the local male population.

Materials and Methods

Participants of the study: The total number of cases studied was 117 and divided into the following age-matched groups: Haria drinking male group (n = 30), Haria drinking female group (n = 15), IMFL drinking male group (n = 20), IMFL+Haria group (n = 20), non-drinking male (n = 16) and non-drinking female (n = 16) control groups. The age group of all the participants varied between 20 to 60 years. The blood samples collected from the participants were analyzed biochemically for total serum cholesterol, serum LDL cholesterol, serum

triglyceride and serum HDL cholesterol levels. Detailed information regarding the nature of the study was made available to the participants and consent was obtained from each participant. Prior ethical clearance certificate was obtained from the Institutional Ethics Committee (ICE) that strictly follows the Indian Council of Medical Research (ICMR)'s ethical guidelines for biomedical research on human participants.

Selection of cases: Only those participants were included in the present study that had no other existing disease conditions known to influence the lipid metabolism. In brief, participants suffering from diabetes mellitus, jaundice, and renal disease over the past six months were not included in the study. The sampling was deferred for two weeks in case of reports of minor illness. The layout of the proforma document for each participant is provided (**Supporting Information, Scheme 1)**. The participants were asked to take their habitual diet including alcohol for at least two weeks prior to sampling as hypertriglyceridemia might disappear temporarily if alcohol consumption were stopped.

Collection of blood samples: A morning sample of venous blood after an overnight fast was collected by a dry and sterile syringe. 5 ml of blood was withdrawn from the antecubital vein of the forearm of the participants. Venous stasis was avoided during blood withdrawal as lipid concentration may increase by as much as 15% during prolonged venous stasis. The serum was used for the estimation of required lipid concentration.

Method for estimation of total serum cholesterol: A kit from the Ranbaxy Laboratories was used to determine the total serum cholesterol. It is based on modified Allain's enzymatic method [20]. Briefly, the hydrogen peroxide formed during cholesterol oxidation reacts with 4-Amino antipyrine and phenol in the presence of peroxidase to produce colored quinonimine dye. The intensity of the color produced is proportional to the cholesterol concentration.

Reagents: The buffer solution contained 3 mmol/L of sodium cholate, 0.82 mmol/L of 4-aminophenazone, 14 mmol/L of phenol, 50 mmol/L of Na_2HPO_4 (disodium hydrogen phosphate), 50 mmol/L of NaH_2PO_4 (sodium dihydrogen phosphate), and 0.017 mmol/L of carbowax 6000. The enzyme reagent contained 33 U/L of cholesterol ester hydrolase, 117 U/L of cholesterol oxidase, 67,000 U/L of peroxidase. The cholesterol standard used was 200 mg/dl.

Procedure of the test: The absorbance of the test and standard against blank were measured on a photo colorimeter with a green filter at 530 nm. The optical density (OD) values at different concentration of cholesterol are shown in the **Figure S1**. The readings were linear up to the concentration of 700 mg/dl. .

Calculation: The total serum cholesterol can be calculated by using the formula below:

$$\text{Cholesterol in mg/dl} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times \text{strength of standard}$$

Method for estimation of HDL cholesterol: Low-density lipoprotein (LDL), very low-density lipoproteins (VLDL) and chylomicrons was precipitated by polyanions in the presence of metal ions to leave the HDL in

solution. The cholesterol content of the supernatant fluid was then determined by the enzymatic method. The precipitating reagent used was phosphotungstate/magnesium. A kit from Ranbaxy laboratories was used for this purpose.

Reagent: Buffer solution, enzyme reagent, HDL cholesterol standard of strength 50 mg/dl, precipitating reagent that contains phosphotungstate reagent, magnesium chloride solution (2 mmol/L), and tris Buffer (10 mmol/L). The buffer solution and the enzyme reagent were the same as used in total serum cholesterol estimation.

Procedure of the test: Readings of the absorbance of test and standard were taken against the blank in a photo colorimeter at 530nm wavelength with a green filter as a function of HDL cholesterol concentration. The OD values at different concentration of HDL cholesterol are shown in **figure S2**.

Calculation: The HDL cholesterol can be calculated by using the formula below:

$$\text{HDL Cholesterol in mg/dl} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times \text{strength of standard}$$

Method for estimation of serum triglyceride (TG): Dr. Reddy's kit was used for the estimation of triglyceride level in serum. It is based on GPO-PAP method, an enzymatic method. Briefly, Triglyceride is hydrolyzed to glycerol and free fatty acids by lipoprotein lipase (LPL). In the presence of ATP and glycerokinase, the glycerol is converted into glycerol - 3 phosphate, which is then oxidized by glycerol -3 phosphate oxidase (GPO) to yield hydrogen peroxide and Dihydroxyacetone phosphate in presence of oxygen. Hydrogen peroxide in the presence of peroxidase reacts with chromogen (4-chlorophenol and 4-amino antipyrine) to form a colored complex. The intensity of the color developed is proportional to the triglyceride concentration, which was measured in a photo colorimeter with a green filter at a wavelength of 530 nm.

Reagents: The enzyme reagent contains enzymes as lipoprotein lipase, glycerol-3 phosphate oxidase, glycerol kinase and peroxidase.

Procedure of the test: The absorbance of test and standard were measured against blank on a photo colorimeter at 530 nm of wavelength with a green filter as a function of different concentration of triglyceride. The OD values against different concentration of triglyceride are shown in **figure S3**. It is observed that the curve in linear up to 1000 mg/dl.

Calculation: The triglyceride concentration can be calculated by using the formula below:

$$\text{Triglyceride concentration in mg/dl} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times \text{concentration of standard}$$

Method for estimation of serum LDL cholesterol: In the absence of a separate estimation method of LDL, an indirect method was used in accordance with the outline of Friedewald formula [21]. The LDL cholesterol was calculated from the estimated values of triglyceride, total cholesterol, and HDL cholesterol which were directly

assayed in the serum by methods described above. The value of LDL cholesterol was calculated as LDL Cholesterol = Total cholesterol - HDL – (TG /5).

Statistical analysis: The statistical analysis viz. standard error of the mean, and analysis of variance was done according to Snedecor and Cochran [25]. In case of the significant effect of 'F' in the analysis of variance, the pairwise comparison of mean was done with the help of critical difference test. The formula used for determination of the variance, co-efficient of variance and analysis of variance have been described (**supporting information, scheme 2**).

Results

The desirable level of total serum cholesterol in a healthy male participant is < 5.2 mmol/L (200 mg/dl), LDL cholesterol is < 2.48 mmol/L (100 mg/dl), serum triglyceride is < 1.7 mmol/L (150 mg/dl) and HDL cholesterol is > 1.05 mmol/L (40 mg/dl). Alterations in these values increase the risk of cardiovascular diseases [22]. The result consists of 117 subjects who are apparently healthy, free from any endocrinal and cardiac disorder, as ascertained by their history and clinical examination.

Effect of types of alcohol on the lipid profile of local male groups

Total serum cholesterol: The estimation of the total serum cholesterol level of the male participants who were regular and heavy drinkers of Haria, IMFL or IMFL+Haria and the non-drinking age-matched control groups are shown as a bar diagram in **Figure 1A**. The serum cholesterol in the Haria group (143.00 ± 3.14 mg/dl, mean ± SEM) was found to be similar to that of the age-matched control group (138.00 ± 5.20 mg/dl) while the IMFL group (188.00 ± 7.67 mg/dl) and IMFL + Haria group (177.00 ± 11.14 mg/dl) showed statistically significant elevated serum cholesterol levels ($p < 0.05$).

Serum triglyceride: The estimation of the serum triglyceride (TG) levels of the male participants of the four groups are shown as a bar diagram in **Figure 1B**. The serum triglyceride in the Haria group (104.00 ± 4.39 mg/dl) was found to be similar to that of the age-matched control group (108.00 ± 3.83 mg/dl) while the IMFL group (167.00 ± 14.68 mg/dl) and IMFL + Haria group (126.00 ± 5.23 mg/dl) showed statistically significant elevated serum triglyceride levels ($p<0.05$).

Serum HDL cholesterol: The estimation of the serum HDL levels of the male participants of the four groups are shown as a bar diagram in **Figure 1C**. The serum HDL cholesterol in the Haria group (42.80 ± 1.02 mg/dl) was found to be similar to that of the age-matched control group (42.34 ± 1.06 mg/dl) while the IMFL group (35.60 ± 1.08 mg/dl) and IMFL + Haria group (35.07 ± 0.77 mg/dl) showed statistically significant decreased serum HDL cholesterol levels ($p < 0.05$).

Serum LDL cholesterol: The estimation of the serum LDL cholesterol levels of the male participants of the four groups are shown as a bar diagram in **Figure 1D**. The serum LDL cholesterol in the Haria group (79.00 ± 3.50

mg/dl) was found to be similar to that of the age-matched control group (74.00 ± 3.68 mg/dl) while the IMFL group (119.00 ± 7.30 mg/dl) and IMFL + Haria group (116.00 ± 11.90 mg/dl) showed statistically significant elevated serum LDL cholesterol levels ($p < 0.05$).

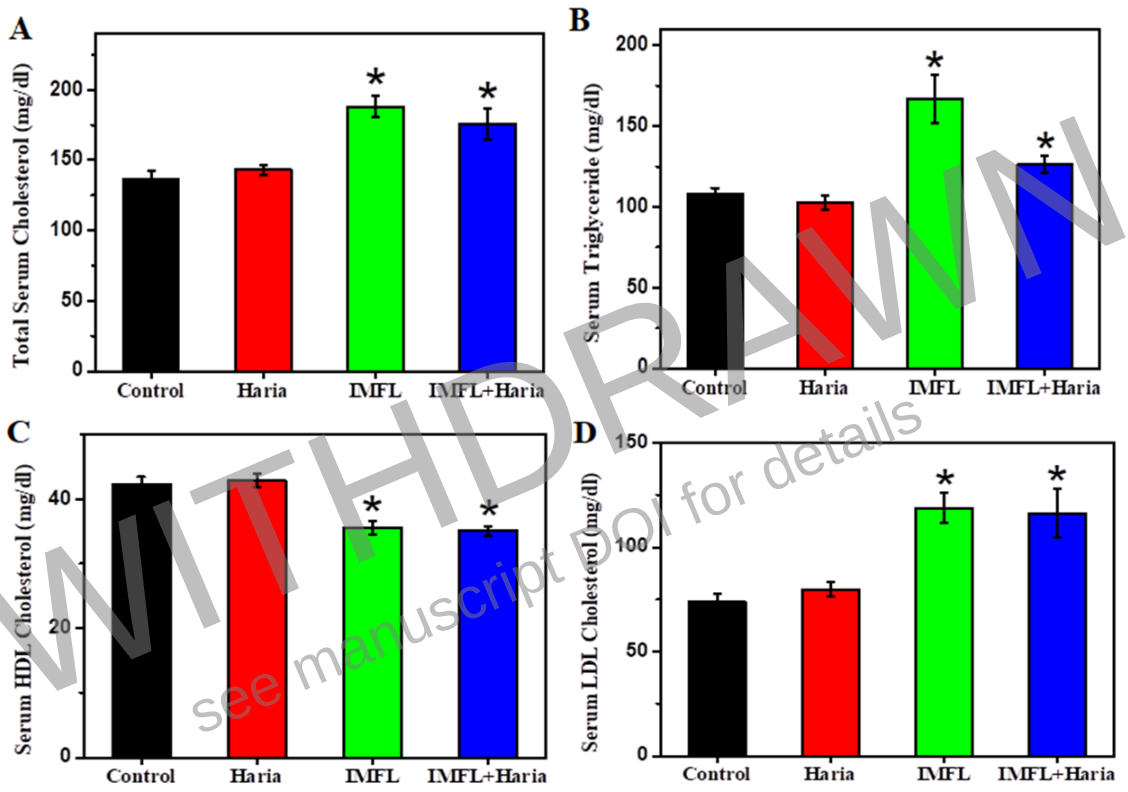

Figure 1. **Effect of the type of alcohol on the blood lipid profile of local male population of Jharkhand**. The local male population was divided into four groups; non-drinking control group (n = 16), Haria drinking group (n = 30), Indian-made foreign liquor (IMFL) group (n = 20) and IMFL + Haria group (n = 20). The blood sample for lipid analysis was collected in the morning following an overnight fasting period. **(A)** The bar graph shows the level of total serum cholesterol concentration, expressed in mg/dl, of the above four groups. The cholesterol concentration of the IMFL (green) and IMFL + Haria (blue) showed significant elevated levels when compared to the controls (black) ($p < 0.05$). The Haria (red) group serum cholesterol levels did not differ significantly from that of the control (black) group. **(B)** The serum triglyceride levels between the control (black) and Haria (red) groups showed no significant difference while a significant elevated levels were observed with the IMFL (green) and IMFL + Haria (blue) groups ($p < 0.05$). **(C)** The serum high density lipoprotein (HDL) cholesterol levels were observed to be significantly lower in the IMFL (green) and IMFL + Haria group (blue) ($p < 0.05$) while no difference was observed in the Haria (red) consuming group when all three groups were compared to the controls (black). **(D)** The serum low density lipoprotein (LDL) cholesterol levels were significantly elevated in the IMFL (green) and IMFL + Haria (blue) group but the Haria (red) group alone showed no significant difference when compared to controls (black). Values represent mean ± SEM.*Indicates that the differences are statistically significant when compared to the control group ($p < 0.05$).

Serum cholesterol/HDL ratio: The estimation of the serum cholesterol/HDL ratio of the male participants of the four groups are shown as a bar diagram in **Figure 2A**. The serum cholesterol/HDL ratio in the Haria group (3.34 ± 0.08) was found to be similar to that of the age-matched control group (3.23 ± 0.12) while the IMFL group (5.28 ± 0.22) and IMFL + Haria group (5.01 ± 0.31) showed statistically significant elevated serum cholesterol/HDL ratio ($p<0.05$).

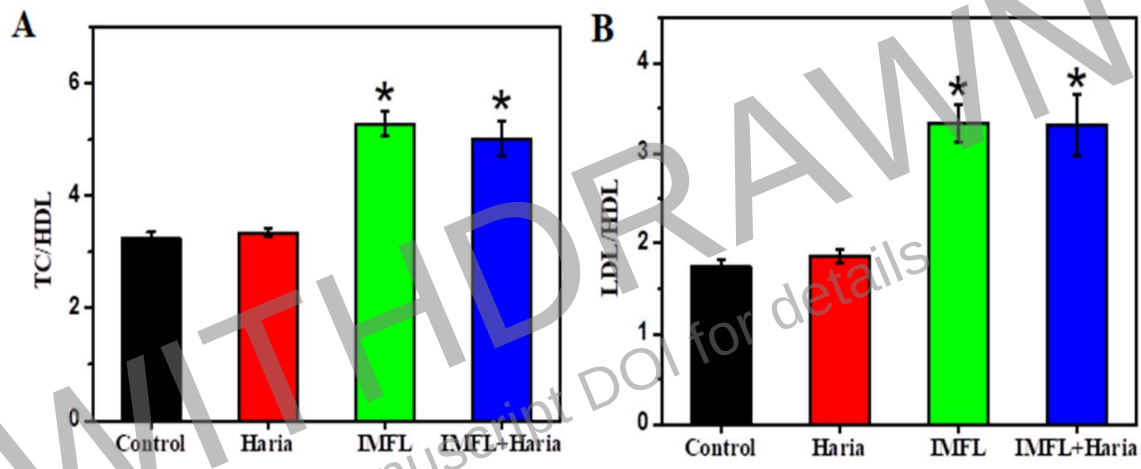

Figure 2. **Effect of the type of alcohol on the blood lipid ratios of local male population of Jharkhand**. The local male population was divided into four groups; non-drinking control group (n = 16), Haria drinking group (n = 30), Indian-made foreign liquor (IMFL) group (n = 20) and IMFL + Haria group (n = 20). **(A)** The bar graph of the ratio of the total cholesterol (TC) to the HDL cholesterol was plotted for various groups. The Haria (red) consuming group shows ratio similar to that of the control (black) group while the IMFL (green) and IMFL + Haria (blue) group shows significant elevated TC to HDL ratio ($p < 0.05$). **(B)** The LDL/HDL ratio showed significant difference when IMFL (green) and IMFL + Haria (blue) groups were compared to the controls (black) ($p < 0.05$). The Haria (red) group behaved similar to the controls. Values represent mean ± SEM. *Indicates that the differences are statistically significant when compared to the control group ($p < 0.05$).

Serum LDL/HDL ratio: The estimation of the serum LDL/HDL ratio of the male participants of the four groups are shown as a bar diagram in **Figure 2B**. The serum LDL/HDL ratio in the Haria group (1.86 ± 0.08) was found to be similar to that of the age-matched control group (1.74 ± 0.09) while the IMFL group (3.33 ± 0.21) and IMFL + Haria group (3.31 ± 0.34) showed statistically significant elevated serum LDL/HDL ratio ($p < 0.05$).

Effect of Haria on the lipid profile of the female group

The female groups of the Dhanbad and Ranchi zone largely consumed Haria; as a result we could carry out the estimation of the blood lipids for the Haria drinking group only.

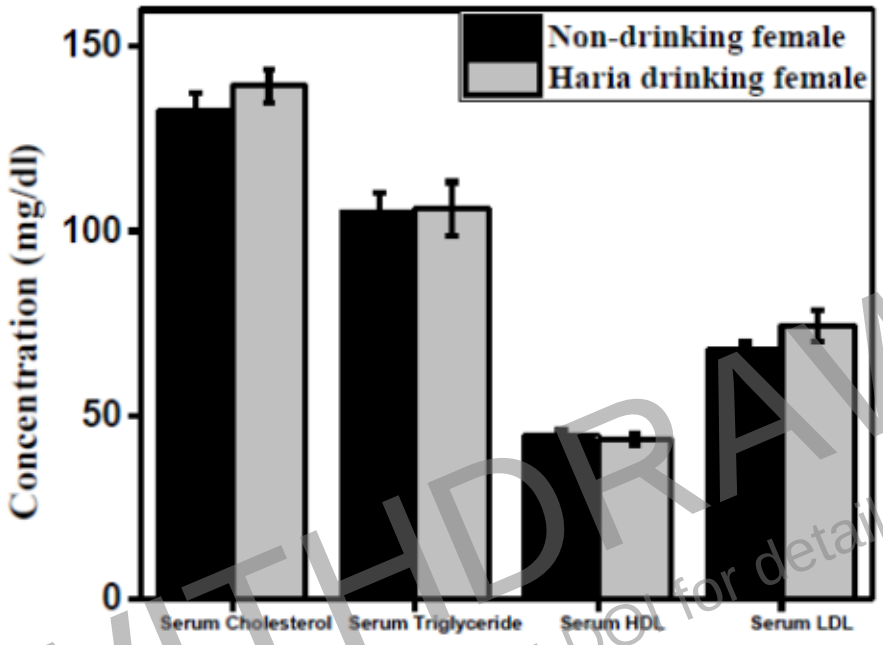

Figure 3: Effect of Haria on the blood lipids of local female population of Jharkhand. The local female population was divided into two groups; non-drinking control group (n = 16) and Haria drinking group (n = 15). The blood sample for lipid analysis was collected in the morning following an overnight fasting period. The bar graph shows the total serum cholesterol, serum triglyceride, HDL and LDL for Haria drinking female (grey) and non-drinking control (black) groups. The Haria consuming group shows no significant difference with the control group in any of the four blood lipid types.

The blood lipid profile of the Haria drinking female groups compared to age-matched control groups are shown in **Figure 3**. The serum cholesterol in the Haria group (139.00 ± 4.54 mg/dl,) was found to be similar to that of the age-matched control group (133.00 ± 5.06 mg/dl). The serum glyceride of the Haria group (105.90 ± 7.30 mg/dl) was found to be similar to the control group (104.90 ± 5.50 mg/dl). The serum HDL cholesterol of the Haria group (43.60 ± 1.50 mg/dl) was found to be similar to the control group (44.30 ± 1.60 mg/dl). The serum LDL cholesterol of the Haria group (74.30 ± 4.40 mg/dl) was also found to be similar to the control group (68.00 ± 2.30 mg/dl).

Discussion

Increased level of total serum cholesterol, LDL cholesterol, serum triglyceride and low level of HDL cholesterol is the leading cause of coronary artery diseases like atherosclerosis such that 1 mg/dl increase in HDL cholesterol accounts to decrease the risk of cardiovascular disease (CVD) by 2% in men and 3% in women [22]. It is estimated that 93% of body cholesterol is located in cells and only 7% circulating in plasma is responsible for atherosclerosis [23]. Several epidemiological findings have also revealed that hypercholesterolemia and hypertriglyceridemia are the important risk factors responsible for the manifestation of atherosclerosis in the life

of individuals. They are influenced by many other predisposing factors such as age, dietary habit, smoking, alcohol intake and habits of the individuals.

Our present study reveals that the regular and heavy drinkers of Haria (both male and female groups) show lipid profile similar to the age-matched and gender-matched control groups. Hence, the popular local rice-based alcoholic beverage appears benign to the detrimental effects of alcohol consumption vis a vis alterations in blood lipid concentrations. Male groups who consume IMFL and a combination of IMFL and Haria shows altered lipid profiles including an increased cholesterol, triglyceride and LDL levels and decreased HDL levels. This is indicative of alterations in physiology that could potentially lead to cardiovascular diseases. Hence these groups are predisposed to disease conditions.

Conclusions

Haria is a popular and heavily consumed local rice based fermented alcoholic beverage of West Bengal, east-central India and parts of Assam. Our findings with the local population of Ranchi and Dhanbad belt of Jharkhand, India, indicate that Haria does not alter the lipid profiles of regular male and female consumers. On the other hand, Indian made foreign liquor and a combination of IMFL and Haria produces chronic alterations to the lipid profiles. Our cross sectional study indicates that there is a crucial link between the types of alcohol consumed and alterations in blood lipids. It will be interesting to investigate the other physiological effects of Haria in future studies and also investigate the underlying mechanisms by which Haria appears to be benign to the detrimental effects of high alcohol consumption. We speculate that Haria contains ingredients that are rich in antioxidants, minerals and other nutrients that could counter the harmful effects of alcohol which requires systematic investigation.

Acknowledgements

The authors would like to acknowledge the infrastructure facility and financial support provided by the Patliputra Medical College and Hospital, Dhanbad, to successfully execute the study. We would also like to thank IIT (ISM) Dhanbad for providing infrastructure facility. We are also grateful to the participants from the Ranchi and Dhanbad zone who willingly took part in this study.

Abbreviations

ARIC, Atherosclerosis Risk in Communities; CVD, Cardiovascular disease; HDL, High density lipoprotein; IMFL, Indian made foreign liquor; LDL, Low density lipoprotein; OD, Optical density; SEM, Standard error of mean; TG, Triglyceride;

Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest.

Research involving human participants and/or animals: The ethical permission was taken from Institutional ethical committee.

Informed consent: Informed consent was taken from the participants who donated blood for this particular study. The format is provided in the supporting information (Scheme 1).

Human and Animal Rights: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Vu KN, Ballantyne CM, Hoogeveen RC, Nambi V, Volcik KA, Boerwinkle E et al. Causal Role of Alcohol Consumption in an Improved Lipid Profile: The Atherosclerosis Risk in Communities (ARIC) Study. PLoS One. 2016;11(2):e0148765. doi:10.1371/journal.pone.0148765.
2. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ. 1999;319(7224):1523-8.
3. Gaziano JM, Buring JE, Breslow JL, Goldhaber SZ, Rosner B, VanDenburgh M et al. Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. N Engl J Med. 1993;329(25):1829-34. doi:10.1056/NEJM199312163292501.
4. Brien SE, Ronksley PE, Turner BJ, Mukamal KJ, Ghali WA. Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: systematic review and meta-analysis of interventional studies. BMJ. 2011;342:d636. doi:10.1136/bmj.d636.
5. Wang C, Xue H, Wang Q, Hao Y, Li D, Gu D et al. Effect of drinking on all-cause mortality in women compared with men: a meta-analysis. J Womens Health (Larchmt). 2014;23(5):373-81. doi:10.1089/jwh.2013.4414.
6. Costanzo S, Di Castelnuovo A, Donati MB, Iacoviello L, de Gaetano G. Alcohol consumption and mortality in patients with cardiovascular disease: a meta-analysis. J Am Coll Cardiol. 2010;55(13):1339-47. doi:10.1016/j.jacc.2010.01.006.
7. Corrao G, Rubbiati L, Bagnardi V, Zambon A, Poikolainen K. Alcohol and coronary heart disease: a meta-analysis. Addiction. 2000;95(10):1505-23.
8. Wakabayashi I. Light-to-moderate alcohol intake reduces lipid accumulation product and attenuates its relation to hypertension. J Hum Hypertens. 2015;29(6):359-65. doi:10.1038/jhh.2014.97.
9. Molina PE, Gardner JD, Souza-Smith FM, Whitaker AM. Alcohol abuse: critical pathophysiological processes and contribution to disease burden. Physiology (Bethesda). 2014;29(3):203-15. doi:10.1152/physiol.00055.2013.
10. Fernandez-Sola J. Cardiovascular risks and benefits of moderate and heavy alcohol consumption. Nat Rev Cardiol. 2015;12(10):576-87. doi:10.1038/nrcardio.2015.91.
11. Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali WA. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. BMJ. 2011;342:d671. doi:10.1136/bmj.d671.
12. Glymour MM. Alcohol and cardiovascular disease. BMJ. 2014;349:g4334. doi:10.1136/bmj.g4334.
13. Bhatti SK, O'Keefe JH, Lavie CJ. Of mice and men: atrial fibrillation in veteran endurance runners. J Am Coll Cardiol. 2014;63(1):89. doi:10.1016/j.jacc.2013.05.103.
14. Crouse JR, Grundy SM. Effects of alcohol on plasma lipoproteins and cholesterol and triglyceride metabolism in man. J Lipid Res. 1984;25(5):486-96.
15. Katsiki N, Tziomalos K, Mikhailidis DP. Alcohol and the cardiovascular system: a double-edged sword. Curr Pharm Des. 2014;20(40):6276-88.
16. Goldberg IJ. To drink or not to drink? N Engl J Med. 2003;348(2):163-4. doi:10.1056/NEJMe020163.
17. Room R, Babor T, Rehm J. Alcohol and public health. Lancet. 2005;365(9458):519-30. doi:10.1016/S0140-6736(05)17870-2.
18. Bagnardi V, Sorini E, Disalvatore D, Assi V, Corrao G, De Stefani R et al. 'Alcohol, less is better' project: outcomes of an Italian community-based prevention programme on reducing per-capita alcohol consumption. Addiction. 2011;106(1):102-10. doi:10.1111/j.1360-0443.2010.03105.x.
19. Volcik KA, Ballantyne CM, Fuchs FD, Sharrett AR, Boerwinkle E. Relationship of alcohol consumption and type of alcoholic beverage consumed with plasma lipid levels: differences between Whites and African Americans of the ARIC study. Ann Epidemiol. 2008;18(2):101-7. doi:10.1016/j.annepidem.2007.07.103.
20. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clinical chemistry. 1974;20(4):470-5.
21. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry. 1972;18(6):499-502.
22. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation. 1989;79(1):8-15.
23. Brown MS, Goldstein JL. Lowering plasma cholesterol by raising LDL receptors. The New England journal of medicine. 1981;305(9):515-7. doi:10.1056/nejm198108273050909.