

Induction of Enzymatic Antioxidants by Moderate Alcohol Consumption

Attequeurrehman M. Harlapur¹, Shashikant V. Nikam², Padmaja S. Nikam³

¹Tutor, Department of Biochemistry, Belagavi Institute of Medical Sciences, Belagavi, Karnataka 590016, India.

²Professor and Head, Department of Biochemistry, Belagavi Institute of Medical Sciences, Belagavi, Karnataka 590016, India.

³Associate Professor, Department of Biochemistry, Belagavi Institute of Medical Sciences, Belagavi, Karnataka 590016, India.

Abstract: *Oxidative stress is implicated in the pathogenesis of atherosclerosis and myocardial infarction. Moderate alcohol consumption has various favourable metabolic changes. In this study Malondialdehyde (MDA) levels and activities of enzymatic antioxidants namely superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were determined in 120 non smoker healthy males with self reported daily consumption of 90ml or 120ml of whisky and rum. 30 non smoker healthy males with no history of alcohol consumption were taken as controls. The result shows significantly elevated levels of MDA ($p < 0.0001$) in participants consuming 120ml of whisky ($6.4 \pm 2.2 \text{ nmol/ml}$) and 120ml of rum ($6.7 \pm 2.0 \text{ nmol/ml}$) compared to those consuming 90ml of whisky ($3.8 \pm 2.0 \text{ nmol/ml}$) and 90ml of rum ($3.9 \pm 1.9 \text{ nmol/ml}$). While the activities of enzymatic antioxidants were significantly increased ($p < 0.0001$) in participants consuming 90ml of alcohol (whisky/rum) compared to those consuming 120ml of alcohol (whisky/rum). Alcohol consumption is associated with dose dependent increase in lipid peroxidation. Moderate alcohol consumption induces defensive antioxidant enzymes; this explains the increase in activities of enzymatic antioxidants in 90ml alcohol consumers. Thus study concludes that consumption of 90ml of alcohol (whisky/rum) in a regularly exercising, non smoker will increase the enzymatic antioxidants. This will lead to reduced oxidative stress which might be the reason for eventual decrease in the risk of cardiovascular disease.*

Keywords: Alcohol consumption, oxidative stress, SOD, GPX, MDA.

1. Introduction

Alcohol consumption on a regular basis and at low volumes that is two standard drinks daily for men which amount to 88.8ml, will provide protection against cardiovascular disease. Whereas regular large consumption in amounts that is more than four to five standard drinks daily which amount to 177.6ml to 222ml daily and heavy episodic drinking of more than four standard drinks are associated with detrimental results.[1]-[6] Alcohol metabolism leads to generation of free radicals causing oxidative stress and increased lipid peroxidation (LPO), which is dependent on the dose of alcohol consumed, these free radicals generated have shown to induce enzymatic antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx) and Catalase (CAT) in animal studies.[7]-[8] Thus the present study was undertaken to evaluate the effect of moderate alcohol consumption on induction of enzymatic antioxidants.

2. Materials and Methods

150 non smoker participants aged 35-55 years with history of regular 30 minutes exercise per day (or equivalent) were included in the study, out of which 120 participants were consuming alcohol daily. They were divided equally into four groups depending on the type and quantity of alcohol consumed into Group I A (90ml whisky), Group I B (90ml rum), Group II A (120ml whisky), Group II B (120ml rum). 30 healthy age matched participants with no history of alcohol consumption served as controls. Ethical clearance from the institute's ethical committee was obtained. Informed written consent was taken from all the participants. The study was conducted in Department of Biochemistry, Belagavi Institute of Medical Sciences, Belagavi.

2.1 Exclusion Criteria

Participants with history of diabetes mellitus, hypertension, tobacco smokers and tobacco chewers, those consuming vitamin and antioxidant supplements and subjects with acute infection and inflammatory disorders were excluded from the study.

2.2 Sample Collection and Assay

5ml of 12 hours fasting venous blood sample was collected under aseptic precaution from the antecubital vein of all the participants. 2ml of the sample was taken in a plain bulb for estimation of MDA levels and 1ml of the sample was taken in EDTA bulb for estimation of activities of enzymatic antioxidants. Serum MDA levels were estimated by method of Satoh K.[9] Activities of enzymatic antioxidants were measured immediately after preparation of hemolysate by using kits from randox laboratories, Ransod for SOD and Ransel for Gpx.[10], [11] Catalase activity was estimated by Aebi H method.[12] The activities of SOD, GPx and Catalase were expressed as U/ml of hemolysate, U/L of hemolysate and catalase units respectively. One catalase unit is mM of H_2O_2 decomposed /mg Hb/min.

2.3 Statistical analysis

All values are expressed as mean \pm SD. Ordinary one-way ANOVA test was employed to test significance between the variables.

2.4 Limitations

The consumption of alcohol was self reported by participants.

3. Results

Table 1 shows comparison of estimated parameters, between controls and participants consuming alcohol and also comparison between the groups consuming alcohol. (Group IA- 90ml whisky, Group IB- 90ml rum, Group IIA- 120ml whisky and Group IIB 120ml rum). The results from the table show significantly lowered MDA levels in participants of group IA/IB when compared with control participants. No significant difference was seen between participants of group IIA/IIB and control participants. MDA levels were significantly higher in group IIA/IIB compared to group IA/IB.

Activities of enzymatic antioxidants (SOD, GPx and CAT) were found to be significantly increased ($p < 0.0001$) in participants of group IA/IB when compared to controls. No significant difference was observed when above parameters were compared between participants of group IIA/IIB and control participants. Group IA/IB showed significantly increased ($p < 0.001$) activities of enzymatic antioxidants compared to group IIA/IIB.

There was no significant difference in estimated parameters when compared between group IA and IB, similarly no significant difference was noted between group IIA and IIB.

3.1 Table

Table 1: Showing comparison of estimated parameters in controls and participants.

Parameter	Controls (n=30)	Group IA (n=30)	Group IB (n=30)	Group IIA (n=30)	Group IIB (n=30)
MDA (nmol/ml)	5.8±2.4	3.8±2 [#]	3.9±1.9 [#]	6.4±2.2 [*]	6.7±2 [*]
SOD (U/ml)	165.2±24.3	199±28.9 [*]	197.3±27.3 [*]	160.5±19.5 [*]	157.2±16.8 [*]
GPx (U/L)	8005±1514	9290±625 [*]	9195±764 [*]	7507±1087 [*]	7368±1012 [*]
Catalase (catalase units)	35.3±4.6	49.4±8.7 [*]	48.6±8.6 [*]	34.0±5.5 [*]	37.9±11.0 [*]

* $p < 0.0001$, [#] $p < 0.001$ = significant, n= Number of participants, all values are expressed as Mean ± Standard deviation.

4. Discussion

Serum MDA is a widely used marker for lipid peroxidation. The mean MDA value was found to be significantly increased ($p < 0.0001$) in both groups of participants consuming 120ml of alcohol, when compared to those consuming 90ml of alcohol (Table 1).

These findings agree with study conducted by Akkus et al (1997) reveals that LPO in the drinkers (measured in terms of MDA) was found to be significantly increased compared to that of controls and was dose dependent.[13] When moderate amount of alcohol is metabolized, it will produce free radicals, in quantity enough to induce synthesis of enzymatic antioxidants as described in different animal studies by Dinu D et al (2005), Gülçin Aykaç et al (1985) and Hurley et al (2012).[14]-[16] The findings of present study shows that in non smoking individuals consuming 90 ml of alcohol (whisky or rum) MDA was not significantly increased as there was significant elevation of antioxidant enzymes. As the dose of alcohol increases LPO will increase as seen in this study where MDA levels were higher in participants consuming 120ml alcohol.

In the present study the activities of enzymatic antioxidants (SOD, GPx and Catalase) were significantly increased ($p < 0.0001$) in both groups of participants consuming 90ml of alcohol when compared to control participants. Significant increase ($p < 0.0001$) was noted in participants consuming 90ml of alcohol on comparison with both group of participants consuming 120ml of alcohol.

Montoliu C et al (1994) and Grasselli E et al (2014) in their study they noted significantly enhanced levels of superoxide dismutase and catalase activities in moderate alcohol consumers compared to MDA levels.[17]-[18] Another study by Lecomte, et al (1994) revealed that the activities of enzymatic antioxidants were elevated in study group

consuming around 59±25.7g of ethanol per day, but as the dose of alcohol (>80g of ethanol) increased the MDA levels increased and activities of enzymatic antioxidants decreased.[19]

The study suggests that increase in the activities of enzymatic antioxidants in participants consuming 90ml alcohol may neutralize the free radicals thereby protecting the biomolecules from free radical injury. However, the activities of enzymatic antioxidants were not significantly increased in participants consuming 120ml of alcohol. These findings may suggest that in participants consuming 120ml alcohol the enzymatic antioxidants are used up to neutralize the substantial amount of free radicals generated. From the results it is evident that consumption of 90ml of alcohol significantly increases the activities of enzymatic antioxidants on comparison with control participants and those consuming 120ml of alcohol. Once the balance of oxidative stress is in the favor of anti oxidants, excess free radicals are neutralized preventing biomolecules from oxidative damage.

5. Conclusion

Daily consumption of 90ml alcohol (whisky/rum) in non smoker individuals will increase the activities of enzymatic antioxidants and thereby reduces oxidative stress.

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Author Profile

Attequeurrehman M Harlapur received his M.B.B.S and M.D degrees from J.J.M. Medical College, Davangere (2007) and Belagavi Institute of Medical Sciences, Belagavi (2015) respectively. After completion of post graduation, He joined Belagavi Institute of Medical Sciences as a Tutor. His field of interest is antioxidants

Shashikant V. Nikam received M.Sc. Medical biochemistry and Ph.D. Medical biochemistry from Government Medical College, Miraj, Maharashtra in the year 1998 and 2005 respectively. He is at present working in Belagavi Institute of Medical Sciences, Belagavi as Professor and Head of Department of Biochemistry.

Padmaja S. Nikam received M.Sc. Medical biochemistry and Ph.D. Medical biochemistry from Government Medical College, Miraj, Maharashtra in the year 1999 and 2009 respectively. She is at present working in Belagavi Institute of Medical Sciences, Belagavi as Associate Professor in Department of Biochemistry.