



**RED WINE INTAKE COUNTERACTS BREWED BEER DISTORTIONS OF CARDIAC MICROSTRUCTURE AND TROPONIN CONCENTRATION IN EXPERIMENTAL MODELS**

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**Abstract**

While cardiovascular dysfunction has become a cause for serious concern with high morbidity and mortality rates worldwide, there is increase in availability and consumption of alcohol-containing products now associated with some health problems. Two different brands of alcoholic beverages (*brewed beer and red wine*) were investigated to determine whether their regular consumption cause any changes in heart microarchitecture and serum concentrations of cardiac troponin I (CTnI) in male experimental models. Twenty albino *Wistar* rats weighing 120 to 200g were allotted to four groups (n=5). Group A received distilled water and served as the control; Group B was administered 5ml/kg body weight per day of beer (containing 5% alcohol by volume, ABV); Group C was given 5ml/kg of red wine (12% ABV); and Group D was administered with the same amount of beer and then followed with red wine in quick succession. Treatments were given orally for 15 days after which animals were euthanized with isoflurane. The thoracic cavity was exposed to collect blood samples from the heart and afterwards, tissue samples also excised and immediately fixed in 10% formalin. Paraffin embedding method was used for tissue preparation while transverse sections were cut at 5 µm and stained with hematoxylin and eosin for histological examination. The blood samples were centrifuged to obtain serum used for (CTnI) assay via Erenna ultrasensitive method. Data obtained was analysed using SPSS Statistics software via One-way analysis of variance (ANOVA), Post hoc and Tukey to evaluate differences in concentration among various groups. The results revealed red wine mitigates alterations in cardiac microstructure following beer intake, with minimal loss of myofibrils, reversal of congested blood vessels and reduced myocardial inflammation; as well as abrogates disruptions in CTnI concentration evidenced from decreased concentration in group D when compared with group B that had the highest mean. These findings indicate that red wine intake at the investigated amount counteracts beer-induced alterations of cardiac microstructure and function.

**Keywords:** Alcohol, red wine, brewed beer, cardiac troponin, histology

## 1.0 Introduction

Alcoholic beverages are divided into three general classes: beers, wines and spirits. These products are frequently consumed in different parts of the world for their stress-dampening or intoxicating effects. Beer is made by fermentation of starch combining yeast and malted cereal starch, especially barley corn, rye, wheat or blend of several grains and usually flavoured with hops (Swami et al., 2014). It is mainly composed of water, but it is also rich in nutrients like carbohydrates, amino acids, minerals, vitamins and polyphenols resulting from a multi-step brewing and fermentation process (Arranz et al., 2012). Hop flowers used as a bittering and flavoring agent contain phenolic compounds, including phenylated flavonoids which have been shown in vitro to have different antioxidant, anti-carcinogenic, anti-inflammatory, oestrogenic, and anti-viral biological activities ((Carvalho et al., 2015; Rehova et al., 2004; Arranz et al., 2012; Gerhauser, 2005).

Alcohol content in regular beers varies between 3% and 6% alcohol by volume (ABV), however some may contain up to 8% alcohol while the energy value ranges between 28 and 73 kcal per 100 ml ((Swami et al., 2014; Misbach et al., 2017). The average yearly beer consumption in Europe, in 2018 was 72 litres per capita, with some countries like Czech Republic, Austria and Germany consuming more than 100 litres per capita per year (The Brewers of Europe, 2019). In Africa, Nigeria was said to reach an average beer consumption of 12.28 litres per person per year which translates into higher volumes of beer consumed per year due to its large population size; followed by Uganda

(11.93 litres) and Botswana (7.96 litres). A summary of related studies has shown moderate consumption (up to 55 g alcohol/day) of beer to have a beneficial effect on non-fatal cardiovascular events (De Gaetano et al., 2016). However, a review by Toma et al. (2017), contradicts such positive indication, suggesting that this protective role may be a confounder, due to the inclusion of former drinkers in the non-drinkers group.

Red wine, on the other hand is made from red grapes, which are actually closer to black in colour. The red colour comes from anthocyan pigments present in the skin of the grapes (Swami et al., 2014). It is composed mainly of water, carbohydrates, organic acids, minerals, alcohol, polyphenols and aromatics (Snopek et al., 2018). The most important polyphenols in red wine are resveratrol, anthocyanins, catechins and tannins (Del Pino-Gercia et al., 2017). Red wine production involves three main processes: pre-fermentation that deals with crushing the fruit and releasing juice, fermentation in which the sugars in the juice are converted into alcohol and carbon dioxide through the addition of yeast, and the post fermentation process where wine is raked off the yeast lees. In red wine making, the pulp, skins and seeds of grapes are kept together after crushing and during all or part of the fermentation, in order to extract colour and flavour (Swami et al., 2014). Red wine intake has been related to a lesser risk for coronary heart disease. Experimental studies and meta-analyses have mainly attributed this outcome to its polyphenol properties (Castaldo et al., 2019).

Ethyl alcohol, also known as ethanol or usually just as alcohol contained in these beverages, is the most consumed drug in human history (Klastsky, 2002). At present, its consumption rates are still very high, with a widespread worldwide distribution, in a global uncontrolled scenario with easy access (WHO, 2010). In fact, there is an increasing consumption in particular groups such as adolescents and young people (Moure-Rodriguez, 2018).

This ethanol misuse at high consumption rates has been reported to cause a variety of health problems, ethanol being the 6th most relevant factor of global burden of disease and responsible for 5.3% of all deaths (Rehm et al., 2009). Ethanol has been described as having a variety of effect in all human body organs either in acute or chronic consumption (Szabo and Lippai, 2014). However, the vast majority of studies elucidating the role of alcohol in cardiovascular and in the global burden of disease relies on epidemiological studies of associative nature which carry several limitations. This is why the cardiovascular benefits of low to moderate alcohol consumption are being questioned and perhaps might have been overestimated.

Besides new evidence associating low and moderate alcohol consumption with decreased risk of cardiovascular disease, several questions remain unanswered related to the concrete amount of safe consumption, the type of alcoholic beverage and the age, sex and genetic/ethnic specific differences in alcohol consumption (Chiva-Blanch and Badimon, 2020). The heart is a muscular organ that is vital for pumping blood through

**2.4 Procurement of test substances:** Brewed beer (Life continental larger beer,

the blood vessels of the circulatory system, while troponins are cardiac regulatory proteins that have proved to be reliable blood markers for identifying a variety of myocardial alterations in humans and animals (Herman et al., 2011; Sharma, 2004). Since the cardiac myocyte is essentially the sole source of cardiac troponin T (CTnT) or troponin I (CTnI), increased serum concentration of either these two proteins serve as a strong indication of myocardial injury. There is less published evidence of the roles of the investigated alcoholic beverages in cardiac structure and function.

## **2.0 Materials and methods**

**2.1 Ethical consideration:** The guidelines/procedures for use of laboratory animals for experimental research were followed accordingly.

**2.2 Animal procurement, care and use:** Male albino Wistar rats aged 9 to 12 weeks were obtained from the breeding facility of the Federal University of Agriculture, Umudike, Abia State, and kept in the animal house of the Department of Anatomy, University of Cross River State. All animals were given standard rat chow and water ad libitum

**2.3 Tools/materials:** These included plastic cages, electronic weighing balance, permanent marker, syringes, medical dissecting set, specimen bottles, centrifuge, reagents (formalin, alcohol, xylene), paraffin wax, embedding mould, rotary microtome, microscope glass slides, tissue stains, distyrene plasticizer xylene (DPX) mountant, coverslips and light microscope.

5% ABV) and Majesty semi-sweet red wine (12% ABV) were purchased from a wineshop

and stored in a refrigerator throughout the study period.

**2.5 Experimental design and collection of samples:** Twenty male rats weighing between 120 and 200 grams were allocated to four groups of five animals each. Group A served as the control. Group B was administered 5 ml/kg body weight per day of beer. Group C received 5 ml/kg of red wine, while Group D received beer and immediately followed with red wine at corresponding doses. Treatments lasted 14 days after which the rats were anaesthetized with enflurane. The thoracic cavity was exposed and blood samples were collected and immediately centrifuged to obtain sera for analysis. Heart tissue samples were collected via excision and fixed in 10% neutral buffered formalin for histological studies.

**2.6 Histological processing:** The fixed heart tissue was dehydrated in ascending grades of alcohol and cleared in xylene, and then embedded in molten paraffin. Afterwards transverse sections of tissue were cut at 5  $\mu\text{m}$  (using a rotary microtome) and stained with hematoxylin and eosin. Tissue was then mounted in DPX and allowed to dry for micrography and histopathological evaluation

**2.7 Serum cardiac troponin assay:** The levels of cardiac troponin I were measured

using the ultrasensitive Erenna immunoassay system, following the procedure described in detail by Todd et al. (2007). In brief, 50 microlitres of serum were combined with 150 microliters of assay buffer containing biotinylated capture antibody-coated microparticles. This mixture was incubated for sixty minutes, after which the microparticles were magnetically separated and washed and afterwards, incubated a second time with 20 microlitres of fluorescent dye-labelled detection antibody. 20 microlitre of elution buffer was added after five additional magnetic separation washes. The eluted detection antibody was removed from the microparticles with a filter plate and the resulting eluate was measured in the Erena instrument system.

**2.8 Statistical data analysis:** The one-way analysis of variance was used to determine the differences in concentration of cardiac troponin among various groups by way of Tukey multiple comparison of means. The values were presented as means  $\pm$  standard deviation (SD) and standard error of mean (SEM) while  $P < 0.05$  was taken to be statistically significant. The Statistical Product and Service Solutions (SPSS) was the package used for the analysis.

### 3.0 Results

**Microstructure:** The following plates are the outcome of the histological investigation

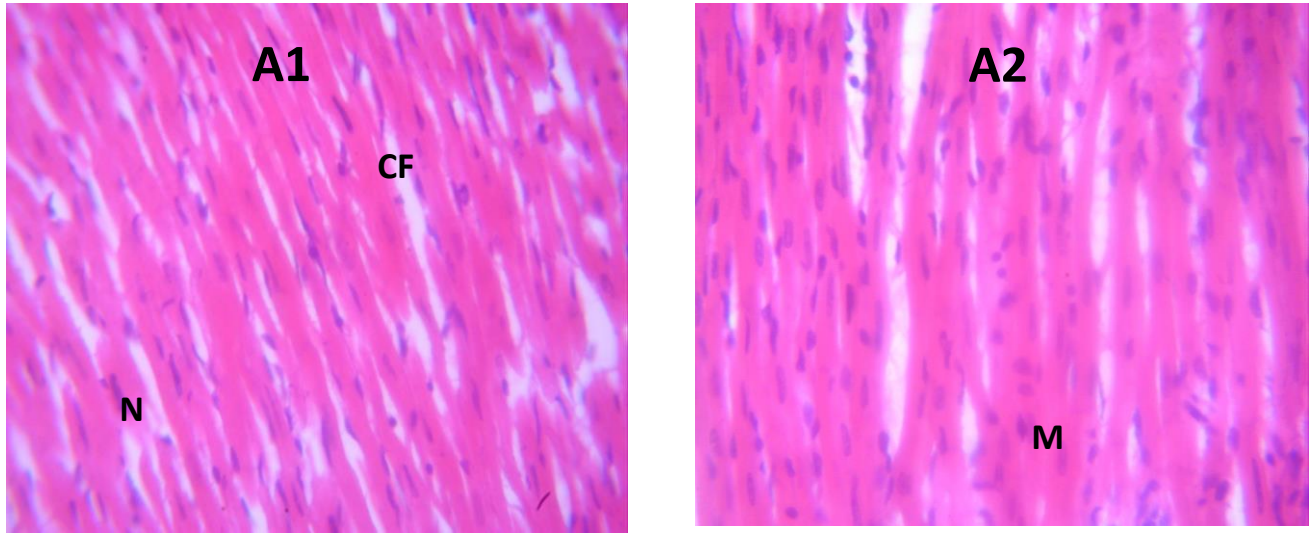


Figure 1: Photomicrographs of samples from group A, control sections of heart (X400) (H/E) show normal cardiac tissue with nuclei (N), cardiac fibre (CF) and cardiac muscles (CM)

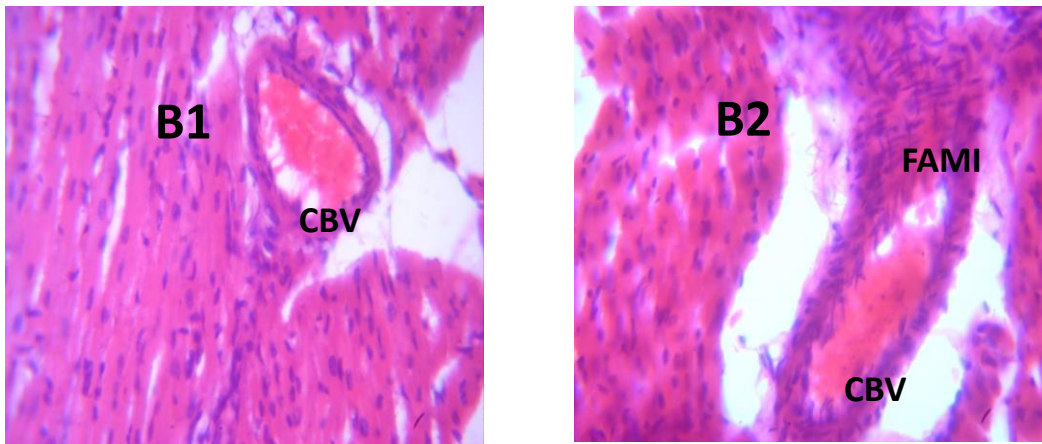


Figure 2: Photomicrographs of samples from group B, sections of heart administered 5 ml/kg of brewed beer (X400) (H/E) show severe alterations with congested blood vessel (CBV) and severe focal aggregate of myocardial inflammation (FAMI) around the CBV in both samples

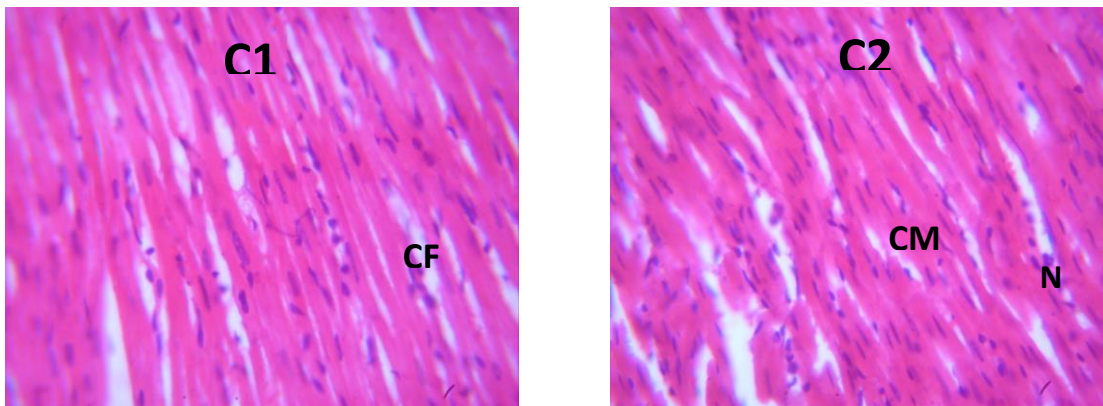


Figure 3: Photomicrographs of samples from group C, sections of heart administered 5 ml/kg of Red wine (X400) (H/E) show well perfused cardiac tissue with normal microarchitecture, visible cardiac fibres (CF), well outlined cardiac muscles (CM) and intact nuclei (N)

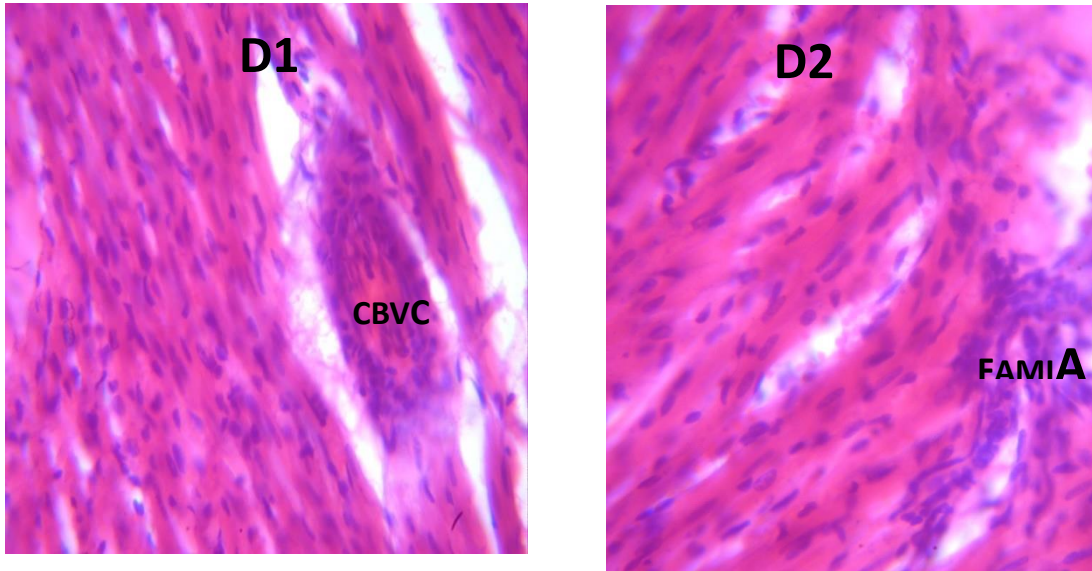


Figure 4: Photomicrographs of samples from group D, sections of heart treated with 5 ml/kg each of both beer and red wine (X400) (H/E) show less distortion of cardiac tissue especially in sample D2 where congested blood vessel (CBV) is inapparent, with mild focal aggregate of myocardial inflammation (FAMI) compared to the severity of cardiac lesions observed in group B samples

**Cardiac troponin assay**

The following tables and chart constitute the output of statistical analysis of Troponin I concentration

**Table 1: Test Descriptive indicate brewed beer had the highest mean**

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	5	4.28	.904	.404	3.16	5.40	3	5
Brewed Beer (BB)	5	5.70	.752	.336	4.77	6.63	5	7
Red Wine (RW)	5	4.12	.942	.421	2.95	5.29	3	5
{(BB) + (RW)}	5	3.66	.956	.427	2.47	4.85	3	5
Total	20	4.44	1.132	.253	3.91	4.97	3	7

**Table 2: ANOVA shows significant difference**

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.620	3	3.873	4.869	.014
Within Groups	12.728	16	.796		
Total	24.348	19			

**Table 3: Multiple Comparisons showing mean difference among various groups**

Dependent Variable: Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Brewed Beer (BB)	-1.420	.564	.095	-3.03	.19
	Red Wine (RW)	.160	.564	.992	-1.45	1.77
	{(BB) + (RW)}	.620	.564	.695	-.99	2.23
Brewed Beer (BB)	Control	1.420	.564	.095	-.19	3.03
	Red Wine (RW)	1.580	.564	.056	-.03	3.19
	{(BB) + (RW)}	2.040*	.564	.011	.43	3.65
Red Wine (RW)	Control	-.160	.564	.992	-1.77	1.45
	Brewed Beer (BB)	-1.580	.564	.056	-3.19	.03
	{(BB) + (RW)}	.460	.564	.846	-1.15	2.07
Brewed Beer + Red Wine {(BB) + (RW)}	Control	-.620	.564	.695	-2.23	.99
	Brewed Beer (BB)	-2.040*	.564	.011	-3.65	-.43
	Red Wine (RW)	-.460	.564	.846	-2.07	1.15

\*. The mean difference is significant at the 0.05 level.

**Table 4: Homogeneous Subsets**

Tukey HSD<sup>a</sup>

Groups	N	Subset for alpha = 0.05	
		1	2
Brewed Beer + Red Wine {(BB) + (RW)}	5	3.66	
Red Wine (RW)	5	4.12	4.12

Control	5	4.28	4.28
Brewed Beer (BB)	5		5.70
Sig.		.695	.056

Means for groups in homogeneous subsets.

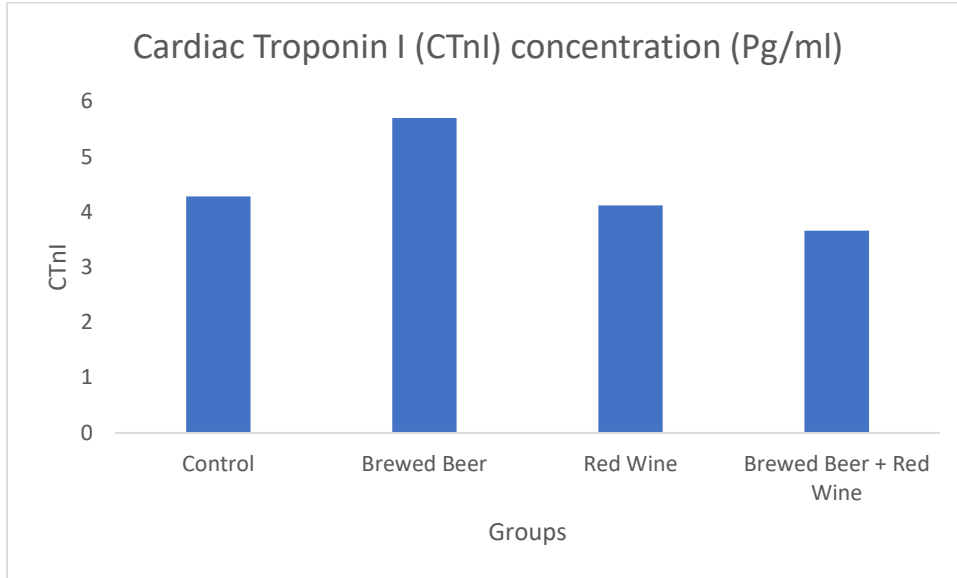


Figure 5: Chart showing the levels of cardiac troponin in various groups

#### 4.0 Discussion

Histological evaluation of sections of myocardium administered brewed beer (figure 2) showed severe alterations with focal aggregate of myocardial inflammation and congested blood vessels. Thus, there was a significant change in morphology when compared to the control group samples in figure 1. Although moderate consumption of alcohol is generally demonstrated to exert a protective action in terms of cardiovascular risk; this property seems not to be beverage-specific due to the possible ability of the various components of alcoholic compounds to mediate peculiar effects in vivo. There is lack of published evidence specifically linking beer intake to cardiac morphology.

However, a meta-analysis of the effects of beer consumption on cardiovascular health with outcome parameters being those related to endothelial dysfunction, showed a significantly higher level of total cholesterol in beer drinkers compared to controls. Similar increased levels were observed in high-density lipoprotein (HDL) cholesterol and in apolipoprotein, while no differences were detected in low density lipoprotein (LDL) cholesterol.

Flow mediated dilation (FMD) resulted significantly higher in beer consumers compared to controls, while blood pressure and other biochemical markers of inflammation did not differ when the specific

beer effect on human cardiovascular health was meta-analysed for the first time (Spaggiari *et al.*, 2020). That study reported an improvement of vascular elasticity, detected by the increase of flow mediated dilation (FMD) after acute intake and of the lipid profile with a significant increase of HDL and apolipoprotein serum levels. The report indicated that although the long-term effects of beer consumption are not still understood, a beneficial effect of beer on endothelial function should be supposed. Photomicrographs of group C, sections of the myocardium administered with red wine in figure 3, showed normal and well perfused cardiac tissue with no significant changes when compared with the control group. Furthermore, samples administered both beer and red wine showed significant reduction of alterations when compared with group B that received beer only. The results from a large cross-sectional study of consumption patterns and cardiovascular disease risk biomarkers, support the hypothesis that heavy alcohol drinking has an effect on cardiac structure and function that may not be driven by atherosclerosis (Lakunchykova *et al.*, 2020).

The outcome of cardiac troponin assay was in support of the alternative hypothesis. The descriptives from statistical analysis in table 1, shows the means and standard deviation values for the three experimental groups: beer, red wine and beer plus red wine to be  $5.70 \pm 0.75$ ,  $4.12 \pm 0.94$  and  $3.66 \pm 0.95$  respectively, while  $4.28 \pm 0.90$  was obtained for the control group. More so, the analysis of variance in table 2 indicates significant difference in concentration of CTnI while the multiple comparisons in table 3 further shows

the mean differences among various groups. The bar chart in figure 5 also shows an increased level for group B that received beer only while there was a decrease in the plot level of group D that was administered both test substances.

Evidence for a direct harmful effect of alcohol on myocardial health using a large cross-sectional study of consumption patterns and cardiovascular disease risk biomarkers from Northwest Russia, 2015 to 2017, showed high sensitivity cardiac troponin T was elevated by 10.3% in support of the hypothesis that heavy alcohol drinking has an adverse effect on cardiac structure and function that may not be driven by atherosclerosis (Lakunchykova *et al.*, 2020). While cardiac troponin I with its high specificity and sensitivity has become the gold standard for detecting acute myocardial infarction, and the cardiac myocyte is essentially the sole source of it, increased serum concentration of this protein serves as a strong indication of myocardial injury (Herman *et al.*, 2011).

Alcohol drinking is increasingly recognized as a risk factor for cardiovascular diseases, and even when consumed in moderation, is associated with complex changes in blood, biochemistry, involving changes in many biomarkers for cardiometabolic risk (Manthey *et al.*, 2018; Wurtz *et al.*, 2016). However, the intake of red wine (though being an alcoholic beverage also) has been related to a lesser risk for coronary heart disease (CHD). Experimental studies and meta-analyses have mainly attributed this outcome to the presence in red wine of a great variety of polyphenolic compounds such as resveratrol, catechin, epicatechin, quercetin

and anthocyanin (Castaldo *et al.*, 2019). Several studies provide evidence that light to moderate alcohol consumption is associated with a higher level of high-density lipoprotein cholesterol (HDL-C), a lower incidence of type-2 diabetes (T2D), and a reduction of lipid oxidative stress (Nova *et al.*, 2019; Golan *et al.*, 2018). Such epidemiological studies have supported that red wine consumption is more CHD-preventative in comparison to the intake of other alcoholic beverages (Torres *et al.*, 2015). Heart disease is the leading cause of death in the US. Moderate red wine consumption has been linked to a reduction in the risk of death by heart disease and heart attack by 30-50%. With about 600,000 people dying from heart disease in the US each year, red wine has become increasingly popular among health-conscious consumers (Higgins and Llanos, 2015).

According to Mostofsky *et al.* (2016), the cardiovascular (and overall) health effects of drinking are both acute and chronic (accumulative) and are strongly determined by the quantity and pattern of alcohol intake, while the acute response to alcohol may also be determined by drinking habits and alcohol tolerance. Lazo *et al.* (2016) posits that moderate alcohol intake is associated with lower levels of high sensitivity cardiac troponin T (hs-CTnT) and N terminal pro B-type natriuretic peptide (NT-pro BNP), whereas abusive intake is associated with increased levels of these biomarkers of cardiac damage. Alcohol has a hormetic physiological behaviour that results in either increased or decreased cardiovascular risk depending on the amount consumed, drinking frequency pattern of consumption and the outcomes under study and even the

type of alcoholic beverage consumed (Chiva-Blanch and Badimon, 2020)

Previous studies reported males to be less sensitive to the adverse effects at a similar level of alcohol consumption (El-mas and Abdel-Rahman, 2019). Besides, intrinsic sex differences in the metabolism and biological effects of alcohol consumption are different between men and women, as the latter tend to drink less amounts of alcohol than men, which in turn may be related to the sensitivity to alcohol (Cheung *et al.*, 2019). In a review evaluating randomised control trials, examining the recent literature on the correlations between acute and chronic Red wine (RW) consumption and health including antioxidant status, cardiovascular function and arterial stiffness, hypertension, immune function, lipid profile and inflammation status; it was reported that RW consumption mostly results in improvements in antioxidant status, thrombosis, and inflammation markers, lipid profile and gut microbiota, with conflicting results on hypertension and cardiac functions (Lombardo *et al.*, 2023).

## 5.0 Conclusion

This research finding indicate that regular consumption of beer at the investigated amount disrupts cardiac microstructure and serum troponin level, whereas moderate intake of red wine can reverse ethyl alcohol-induced cardiac alterations.

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