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Research Article

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Evaluation of trace quantities of cobalt (II) ion concentrations in beer and beer drinkers (human serum).

*Emeji, Roseline[1] and George, Daye Mandy.C.[2]

[1]School of Medical Laboratory Sciences, Rivers State College of Health Science and Technology, P.M.B 5039, Nigeria.

[2]School of Pharmacy Technicians, Rivers State College of Health Science and Technology, P.M.B 5039, Nigeria.

Abstract

The study was done to investigate the degree of exposure to cobalt through the consumption of 10 different brands of commercial beer widely consumed in Nigeria. A total of 40 volunteered individuals comprising 20 heavy beer drinkers (10 males and 10 females), who took at least three bottles per day, and 20 non beer drinkers (10 males and 10 females) were investigated. 5 ml venous blood was collected from each group of individuals using sterile syringe and needle, and the blood digested accordingly. Cobalt levels were tested in Solar Thermo Elemental atomic absorption spectrophotometer model S4-71096 at chromatic wavelength of 380nm. The results showed that cobalt concentrations found in the two batches of beer produced between May and August varied between 0.021µg/L -0.297µg/L and 0.020µg/L -0.299µg/L respectively. However, they were found to be within the permissible limit by law. At 95% confidence level, using F distribution in a single factor anova, there were significant differences in cobalt levels between heavy Male drinkers and non drinkers; a similar trend also existed between the heavy female drinkers and non drinkers. The result was the same for heavy female beer drinkers and heavy male beer drinkers. However, the level of cobalt obtained cannot lead to the risk of suffering cardiomyopathy if beer drinkers are advised to reduce their consumption rate over time. The method was simple, less expensive, uncomplicated, and reproducible and was found to give good results.

Keywords: *Cobalt; beer consumption; and serum cobalt level*.

I. Introduction

Cobalt (Co) is a naturally occurring element that has properties similar to those of iron (Fe) and nickel (Ni) [1]. It is a hard lustrous gray metal, with the symbol Co and atomic number 27. Naturally, it is found only in combined forms in meteorites, rocks, soils, water, plants and animals typically in some amount [2]. Cobalt has 8 isotopes out of which only one is stable [3].Cobalt metal is usually mixed with other base metals to form alloys, which are harder or more resistant to wear and corrosion e.g Cobalt- Chromium- Molybdenum alloys used for prosthetic parts such as hip and knee replacement. Similarly, for dental prosthetics, alloys of iron-cobalt are used [4].

Industrially, these alloys are also used in a number of military, industrial applications such as aircraft engines, magnets, grinding and cutting tools. They are also used as colorant in glass and ceramics, and as trace additives in agriculture [5]. Medically, Cobalt is used to treat refractory anemia (up to 50mg/day) which is not close to the amount ingested in beer (up to 100g/kg). However, studies have shown that the heart is very sensitive to cobalt and can lead to cardiomyopathy (i.e abrupt on set of left ventricular failure) [6]. In the brewing industries, cobalt salts are added to beer to stabilize foaming or frothing. In the 1960s, some breweries therefore added cobalt salts to beer, resulting in exposure of 0.04 – 0.14mg cobalt/kg.

Beer drinking is a social habit and people all over the world drink beer either for pleasure, as a part of social life style or an addiction. Excessive beer drinking is universally accepted as one of the causes of hematological abnormalities. Alcohol as found in beer, contains a lot of toxic and carcinogenic chemicals such as gum Arabic (acacia), sodium hydrosulfite ($Na_2S_2O_2$), tannic acid (tannin), potassium metabisulfite, tartaric acid ($H_2S_4O_6$), Papain (papayotin), magnesium sulfate ($MgSO_4.7H_2O$), dextrin and cobalt. These chemicals also have a narcotizing action on the central nervous system [7]. Some people who drink excessive amounts of beer experienced serious effects on the heart which results in death in some cases. Nausea and vomiting were usually reported before the effect on the heart was noticed. Other ill effects include hip pains, dying tissue, declining cognitive function, mental impairment, hearing and sight defects, seizures, and other multiple heart problems.

There has been growing concern about the role of cobalt in biological and environmental systems. Normally, small amount of cobalt is essential for oxygen transportation and enzymatic activation, respectively, in all animals. But excess in take or huge dose exposure of cobalt in any form is very toxic both to plants and animals. Hence, quantification of various biological samples for cobalt is very important to know their influence in the system. Upon the backdrop of the implications of cobalt in the health of individuals, this study was designed to evaluate trace quantities of cobalt in Nigeria brewed beers, and establish if their levels are of acceptable levels and if beer consumption can increase cobalt serum levels.

The specific objectives of the study are to ascertain:

(1) If the cobalt content of Nigeria brewed beers are of acceptable limit.
(2) If the serum cobalt level had any significant difference between beer drinkers and non beer drinkers.
(3) If there is any significant difference in cobalt serum levels between female and male beer drinkers.

II. Methodology

Study Area

The study area is Port Harcourt metropolis, the capital city of Rivers State of Nigeria, which host the two major refineries of the country. It is highly industrialized and had other allied companies. Night life is a common feature during which much beer is consumed, apart from consumption after work.

Materials

A total of 40 individuals were used for this study, which were made up of 20 individuals (10 males and 10 females) heavy drinkers of beer, (i.e greater than 6 glasses of beer per day) while the remaining 20 other individuals were made up of (10 males and 10 females) non beer drinkers.10 different brands of Nigeria's locally brewed beer were also obtained for this study. The brands include: Star, Heineken, Gulder, Dubic, Grand, "33", Harp, Eagle, Satzenbrau and Castle beer.

Experimental

Procedure for Blood Collection

Venial blood was collected by venal puncture from the femoral vein in the volunteers using sterile 5ml syringe and needle. The collected blood samples were delivered or transferred into dry plain specimen bottles. The blood was allowed to clot, retracted and centrifuged at 3000rpm for 5 minutes. Each serum sample was carefully transferred into another well dried, labeled sample container by a clean Pasteur pipette. 10 ml of each beer sample was also dispensed into plastic containers.

Procedure for Beer Sample Analysis

Digestion of the beer sample was carried out by transferring 100ml of each beer sample into a set of 10 beakers, followed by the addition of 2ml nitric acid and covering of the mouth of the beakers with a watch glass. The contents the beakers were brought to a slow boil and evaporated on a hot plate to the lowest volume of about 10 to 20ml. The beakers were the rinsed with about 10ml of distilled water.

Digestion was completed when the colored solution in the beaker became light in color and very clear. The solution was filtered and the filtrate collected into 100ml volumetric flasks and the volume made up to the 100ml mark with distilled water and mixed thoroughly. Portions of the solution in the flasks were aspirated with AAS for the required metal determination and the result read out directly.

Procedure for Blood Sample Analysis

Digestion of the blood was carried out by transferring 2ml of blood sample collected from the volunteers into a set of 40 beakers containing 20ml of distilled water, followed by the addition of 5ml nitric acid and 2ml perchloric acid. The mouth of the beakers was covered and the contents evaporated slowly on a hot plate until about 10 to 15ml of the content remained. 10ml of distilled water was used to rinse the beaker and digestion was completed when a clear light yellowish solution in the beaker was observed. The filtrate was then filtered into the 25ml volumetric flasks and the volume made up to 10ml or 15ml with distilled water and mixed thoroughly. A portion of this solution was aspirated with AAS for the required cobalt metal determination and the result read out directly.

III. Results

Table1: Cobalt concentrations found in different brands of beer measured in May and August

S/N	Names of Beer Samples	Cobalt Levels (µg/L) in Beer Produced by May	Cobalt Levels (µg/L) in Beer Produced by August
1	Satzenbrau	0.049	0.060
2	Gulder	0.089	0.113
3	Harp	0.030	0.029
4	Star	0.069	0.021
5	Dubic	0.021	0.020
6	Grand	0.026	0.030
7	Heineken	0.114	0.109
8	"33"	0.110	0.121
9	Eagle	0.267	0.270
10	Castle	0.297	0.299

Table 2: Results of cobalt levels in ten different brands of beer tested between May and August with statistical evaluation (n=10).

Sampling periods	Mean ± SD	Co-efficient of Variance (CV).	Standard Error (SR).	Skewness
May	0.1072 ± 0.09819	0.91597	0.03105	1.3238
August	0.1072 ± 0.1015	0.94752	0.03212	1.1963

Table 3: Cobalt concentrations found in human serum.

S/N	Names of Beer Samples	Male Beer Drinkers(*µg/L*)	Female Beer Drinkers(*µg/L*)	Male Non- Beer Drinkers(*µg/L*)	Female Non- Beer Drinkers(*µg/L*)
1	Satzenbrau	0.241	0.048	0.024	0.022
2	Gulder	0.289	0.157	0.023	0.016
3	Harp	0.147	0.147	0.020	0.015
4	Star	0.432	0.059	0.013	0.023
5	Dubic	0.261	0.234	0.022	0.014
6	Grand	0.341	0.169	0.014	0.018
7	Heinekin	0.307	0.278	0.021	0.020
8	"33"	0.369	0.081	0.020	0.015
9	Eagle	0.253	0.170	0.014	0.013
10	Castle	0.412	0.240	0.024	0.019

Table 4: Analysis of variance for male beer and non beer drinkers

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F(MSR)*	*P-value*	*F crit*
Between Groups	0.40812245	1	0.40812245	109.4910596	4.4254E09	4.413873405
Within Groups	0.0670941	18	0.00372745			
Total	0.47521655	19				

SS: Sum of squares, DF: Degree of freedom, MS: Mean square and MSR: Mean square ratio.

Table 5: Analysis of variance for female beer and non beer drinkers

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F(MSR)*	*P-value*	*F crit*
Between Groups	0.0991232	1	0.0991232	32.30955442	2.16577E-05	4.413873405
Within Groups	0.0552226	18	0.003067922			
Total	0.1543458	19				

SS: Sum of squares, DF: Degree of freedom, MS: Mean square and MSR: Mean square ratio.

Table 6: Analysis of variance for male and female beer drinkers

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F(MSR)*	*P-value*	*F crit*
Between Groups	0.40812245	1	0.40812245	109.49106	4.4254E-09	4.413873405
Within Groups	0.0670941	18	0.00372745			
Total	0.47521655	19				

SS: Sum of squares, DF: Degree of freedom, MS: Mean square and MSR: Mean square ratio.

IV. Discussion

Comparison of Different Brands of Beers Brewed Between Two Periods of The Year.

The result in table1 showed variations in the concentration of cobalt in the ten beer brands studied. Lower cobalt concentrations were found in Satzenbrau (0.049µg/L), Gulder (0.089µg/L), Harp (0.030µg/L), Star (0.069µg/L), Dubic (0.021µg/L) and Grand (0.026µg/L). While higher concentrations of cobalt were found in Heineken (0.114µg/L), "33" (0.110µg/L), Eagle (0.267µg/L) and Castle (0.297µg/L) respectively. This implies that higher levels of cobalt could be ingested by consuming more bottles of Heineken, "33", Eagle and Castle (table 1). As you consume more of the aforementioned beer brands, there is likely to be an increase of cobalt levels in the blood serum of the consumer than brands like Satzenbrau, Gulder, Harp, Star, Dubic and Grand. However, all beer brands when consumed expose consumers to certain levels of cobalt apart from food and drugs.

Again, table (1) reveals that the cobalt concentrations in the ten brands of beer evaluated between May and August respectively do not vary in such a way that exceeded permissible limit (0.020-0.3µg/L). It can therefore be inferred that beers brewed in Nigeria are all below the permissible limit, implying that the brewers kept or maintained standard. The obtained values were consistent with international recommendation of less than 0.3µg/L as reported by World Health organization. Thus, cobalt levels in Nigeria brewed beers are of acceptable limit [8].

Validation of the Method Used.

In order to validate the method used to obtain cobalt concentrations in the two different periods (May and August) of the year studied, the data were subjected to descriptive statistics (Table 2). The mean ± SD, coefficient of variance and standard error for the monitored periods were observed to be 0.1072 ± 0.09819, 0.91597 and 0.03105 for May and 0.1072 ± 0.1015, 0.94752 and 0.03212 for August respectively. The result revealed that the standard deviation, coefficient of variance and standard error about the mean were very small and close to each other and comparable, indicating the accuracy of the proposed method of assessment. Validation is further confirmed by the low values of standard deviation, coefficient of variance and standard errors and the skewedness of both periods in the same direction [9], thus implying that the method used was correct, precise and simple.

The result in table 3 reveals that there was variation of cobalt concentrations in blood serum of both heavy beer drinkers and non beer drinkers. The blood serum cobalt levels varied between 0.147-0.412µg/L in male beer drinkers and varied between 0.048-0.278µg/L in female beer drinkers. Variation also existed between male non beer drinkers (0.013-0.024µg/L) and female non beer drinkers (0.013-0.023µg/L) respectively. This result indicates that blood serum cobalt levels was higher in male beer drinkers than in female beer drinkers and a similar trend is observed in male non- beer drinkers than in female non- beer drinkers. All these values did not exceed the recommended levels of 0.11-0.045µg/L in the blood.

This implies that beer can affect serum cobalt levels, and habitual drinking can expose an individual to an increased serum cobalt levels, which if unchecked could snow ball into some medical complications such as cardiomyopathy [10,11];derangements of endocrine (i.e effect on thyroid glands) and abnormal electrocardiographic changes [6,12]. The high levels of serum cobalt level could also be attributed to inadequate intake of sulfhydroxyl group containing amino acid (protein) and vitamin in their foods especially thiamine and zinc depletion [1].

Finally, to ascertain whether there are significant differences in the cobalt levels in beer drinkers and non beer drinkers (Male and females), statistical analysis of variance for a single factor experiment, using F- distribution, was carried out on both drinkers and non drinkers, respectively. Tables 4, 5 and 6 are analysis of variance tables for male (beer and non beer drinkers) , female (beer and non beer drinkers), and male and female (beer drinkers) respectively. From the anova table (4), it can be seen that the sum of squares between and within groups is 0.40812245 and 0.067041 respectively. This gives mean square values of 0.40812245 and 0.00372745 respectively. Similarly, table 5 gave sums of squares between and within groups as 0.0991232 and 0.0552226; with mean squares of 0.0991232 and 0.003067922 respectively. A similar trend existed in table 6 where the sum of squares between groups is 0.40812245 and within group is 0.0670941 with mean square of 0.40812245 and 0.00372745 respectively. At 95% confidence level, the MSR (F) calculated for the male beer and non beer drinkers are 109.4910596, while the tabulated value ia 4.413873405. Similarly, at the same confidence level, the MSR calculated for the female beer and non beer drinkers is 32.30955442 ,while for male and female beer drinkers is same and the tabulated value remained as 4.413873405. Since in the three cases, the calculated MSR is greater than the tabulated values, there are significant differences ($p<0.05$) in serum cobalt levels in beer drinkers and non beer drinkers; so also there is significant difference in male and female beer drinkers, based on the data analyzed at 95% confidence levels.

V. Conclusion

From this study, it can be concluded that Nigerian brewed beers contains cobalt levels that are acceptable all over the world (i.e within acceptable levels). However, beer drinking is a major source of cobalt exposure to man despite medical and dietary sources. Again, the more one drinks beer, the more the serum cobalt tends to raise, which if not checked can make the heavy beer drinkers predispose to medical conditions that could lead to death. Hence, the proposed methods could be employed for routine analysis of cobalt composition in both drinkers and non- drinkers alike. The method is also simple, precise, environmentally friendly and reproducible.

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Conflicts of interests

The authors declare no conflict of interests, thus are unanimous in their respective views.

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