Analysis of Methanol Content in Different Varieties of Traditionally Fermented Alcohol Found in Chandannath Municipality of Jumla District, Nepal

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ABSTRACT

Background: Alcohol poisoning associated with traditionally fermented alcoholic beverages have been reported in Nepal with the constituents of these beverages not being quantified usually. In traditionally fermented alcoholic beverages, methanol, due to its cheaper cost and similar physiochemical properties with ethanol, is adulterated most commonly and readily. The objective of the present study was to quantify four different varieties of traditionally fermented alcoholic beverages available in Chandannath, Jumla for methanol level.

Methods: The present study is a cross-sectional study in which four different types of traditionally fermented alcoholic beverages were collected from Chandannath, Jumla and they were analyzed for methanol content. From Chandannath municipality, the ward number two was selected for collection of samples. Further, from this ward, four different households regularly involved in brewing local alcohol were also randomly selected. These households belonged to three different ethnic groups (Indo-Aryan, Tibeto-Burman and Newar) and these samples were chhyang (sample- 1), local raksi (sample- 2), nigar (sample- 3) and local apple cider (sample- 4). In air tight containers, the samples were sealed and transported to Zest laboratory, Bhaktapur, Nepal for quantification of methanol using Gas Chromatography.

Results: The residents in half of these four households belonged to the Tibeto-Burman ethnic groups. Methanol was detected in two samples among the four analyzed beverages. These samples, sample- 2 and sample- 4, on quantification showed the concentrations of 10.050ppm and 13.721ppm of methanol respectively.

Conclusion: Among four samples of locally brewed alcoholic beverages, methanol was detected in two samples, but at a concentration below the level that would be considered toxic. This finding emphasizes the importance of conducting larger-scale quantification of traditionally fermented alcohol to mitigate the various health risks associated with its potential toxicity, as these beverages are currently not being quantified.

Keywords: Alcohol, alcoholic constituents, gas chromatography, methanol, methanol toxicity.

INTRODUCTION

According to World Health Organization (WHO), every year 2.5 million deaths are recorded due to alcohol toxicity.[1] The alcohol that is consumed can consist of homemade or illicitly produced beverages, as well as smuggled or adulterated products that are not fit for human consumption.[2] Various harmful substances have been identified in many cases among the illicitly produced as well as in surrogate alcoholic products not meant for consumption.[2] Toxic alcohol, a major cause of mortalities and morbidities worldwide, is mostly referred to methanol, isopropanol and ethylene glycol with the mostly adulterated toxic component being methanol and its consumption is.[3,4] Pure form of methanol is lethal at an estimated amount of 1-2ml/kg.[2] Consumption of methanol can lead to toxicity ranging from permanent blindness (with 10ml ingested) to fatal consequences when taken in a dose of 30ml.[1] Results of studies conducted in many parts of Asia, Africa and South America have suggested an increasing incidence of complications associated with methanol adulteration of traditionally fermented alcoholic beverages.[5]

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Methanol is an organic solvent used mostly as a chemical reactant, a paint remover, an industrial solvent, photocopying fluid, windshield washing fluid as well as a source of fuel.[3] Although highly toxic to humans, methanol can be found in the human body, with detectable levels in blood and breath.[6,4,7] The primary sources of methanol exposure are fruits, vegetables, and alcoholic beverages, particularly those derived from fermented fruit distillates.[6,4,7]Methanol is produced during the process of alcohol fermentation. During the distillation process, methanol is distilled alongside ethanol because of their similar physical and chemical properties.[8] As a result, all alcoholic beverages obtained from fermentation contain varying amounts of methanol.[8] In alcoholic beverages, methanol is also produced by the fermenting microbes or another possible source can be raw material's size and age, sterilization temperature, pectin content and pectin methyl esterase (PME) activity.[5,8] Toxicity associated with methanol is primarily due to ingestion but prolonged inhalation or skin absorption can also lead to the same.[3] Also, due to rapid absorption from the gastric mucosa, maximum concentration is achieved within 30 – 90 minutes following consumption.[3]

The toxic impact of methanol can be attributed to two main mechanisms: direct depression of the central nervous system and neurotoxic effects caused by formic acid, a metabolic product derived from the oxidation of formaldehyde, which is itself an oxidized product of methanol.[3,9,6] Formaldehyde is genotoxic and carcinogenic and its oxidized product formic acid leads to metabolic acidosis and a plethora of metabolic derangements.[3,9,6] The above mentioned toxic metabolites of methanol act selectively on retina, optic nerve and central nervous system leading to irreversible damage in these tissues.[9,10]

With regards to Nepal, alcoholic beverages are manufactured traditionally as well as industrially. Traditionally fermented alcoholic beverages are usually not quantified; hence it is very important to determine concentration of methanol in traditionally brewed alcoholic beverages. Gas Chromatography (GC) is the gold standard method for determination of methanol in alcoholic beverages.[11] The present study aimed on detection and quantification of methanol in traditionally fermented local alcoholic beverages at Chandannath, Jumla.

MATERIALS AND METHODS

The study was a cross-sectional study conducted in Chandannath, Jumla, Karnali Province, Nepal from June, 2022 – November, 2022. Jumla district of Karnali Province is located in upper hill region of Mid-Western Nepal. It consists of one municipality (Nagarpalika), i.e, Chandannath and seven rural municipalities (Gaunpalika). In this study, Chandannath municipality was selected by convenience method. Chandannath municipality consists of ten wards. Of these wards, ward number two was chosen. In this ward, four different households were randomly selected for the collection of samples after fulfillment of inclusion and exclusion criteria. Traditionally fermented common alcoholic beverages produced in Jumla from the selected households with three different ethnic groups (Indo-Aryan, Tibeto-Burman and Newar) were included. Likewise, industrially manufactured alcoholic beverages and beverages produced outside Jumla were excluded. The selected households were located at different areas of the ward and were involved in brewing alcohol locally.

The ethical clearance was obtained from the institutional review committee at Karnali Academy of Health Sciences (Ref no: 2078/2079/36). From each of the four selected households, one sample of home brewed alcoholic beverage was collected. Prior to collection of samples from each household, informed written and verbal consent were obtained.

These samples were then transported to a laboratory at Bhaktapur, Nepal. This laboratory (Zest Laboratory and Research Center Pvt. Ltd.) is specialized in the analysis of food, drug, pharmaceutical products amongst others. Using a Gas Chromatography (GC), the contents of methanol in the collected samples were determined.

In the laboratory, all four samples were analyzed to determine the concentration of methanol. The gas chromatography (GC) system used in the present study was manufactured from the United States and consisted of a DB-Wax column with dimensions of 40m x 180μm x 0.3μm. The flow rate for the analysis was set at 0.6ml per minute, and a flame ionization detector (FID) was employed as the detection method. The sample solution was injected into the GC and it entered a gas stream that transported the sample into a separation tube known as the column where various components were separated. The unknown concentration of methanol in the provided samples was measured by injecting standard reference solution whose concentration was known.

RESULTS

This study, conducted in ward number 2 of Chandannath municipality, Jumla, comprised of four households as the study sites from where a total of four locally brewed alcohol samples were collected and analyzed for methanol content. The four different households selected in this study were located in different parts of ward number two. Alcohol samples were collected in an air-tight containers. Using proper safety measures, 500 ml of each sample was collected in separate containers which were marked as samples 1–4. Among the four different types of alcohol, Chhyang (Sample-1) was prepared from kodo (millet), Local raksi (Sample- 2) was prepared from apple, Nigar (Sample- 3) was prepared from rice and Local apple cider (Sample- 4) was prepared from apple.

Table 1: Main ingredients and concentrations of methanol in alcoholic samples

Alcoholic Beverage	Sample Codes	Ingredient	Methanol (ppm)
Chhyang	Sample- 1	Kodo Millet	Not detected
Local Raksi	Sample- 2	Apple	10.050
Nigar	Sample- 3	Rice	Not detected
Local Apple Cider	Sample- 4	Apple	13.721

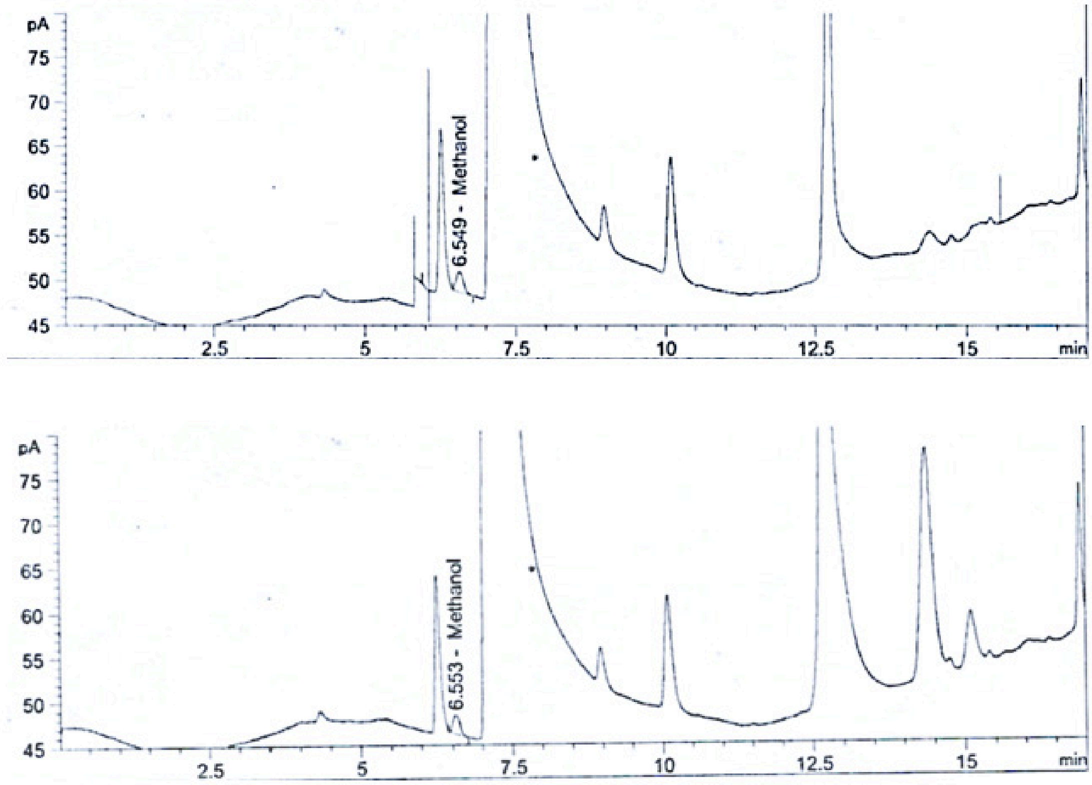

Figure 1: Chromatographic peak value of methanol in sample- 2(A: above; B: below)

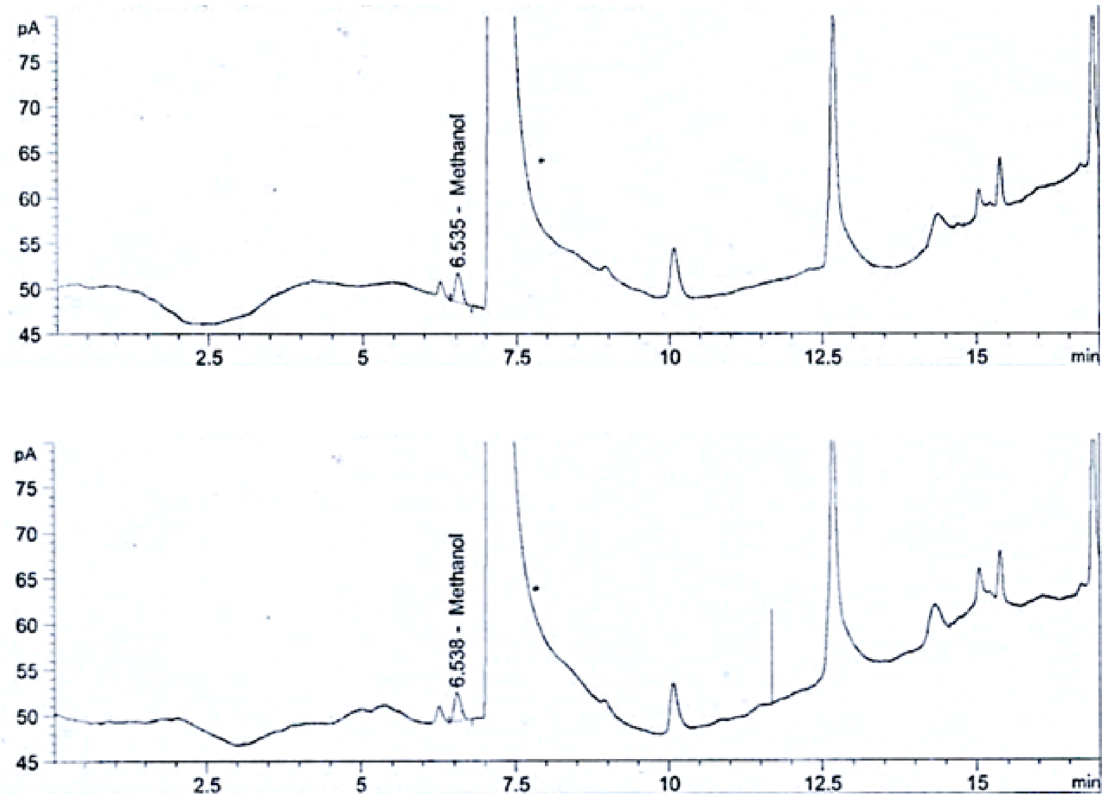

Figure 2: Chromatographic peak value of methanol in sample- 4 (A: above; B: below)

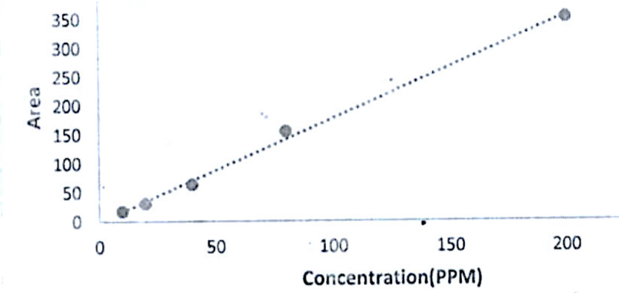

Figure 3: Standard curve of methanol

Table 2: Concentration and area of the curve of standard methanol

Concentration of Methanol (PPM)	Area
9.97	17.74
19.94	31.1
39.98	63.66
79.76	155.13
199.94	351.37

A summary of the main ingredients of all four samples of traditionally fermented alcoholic beverage and its methanol constituent have been stated in table 1.

The peak for each sample was obtained twice in chromatogram. The sample 1 and 3 did not show any rise in peak in the chromatogram and thus methanol was not detected. However, the samples 2 and 4 showed a rise in peak in the chromatogram as shown in figure 1 and 2 respectively. This rise in peak in the chromatogram detected the presence of methanol in samples 2 and 4.

With the average peak retention time of the samples 2 and 4, the area of the curve was obtained. This area of the curve was then used to calculate the methanol concentration from the standard curve of methanol (figure 3).

The standard curve of methanol was plotted from the five certified reference standards of methanol obtained from Sigma Aldrich. The values of the concentration and the area of the standards of methanol based on which the curve had been plotted are mentioned in table no. 2.

In this standard curve of Methanol, the x- axis is the concentration of methanol in parts per million (ppm) and the y- axis is area of curve of peak retention time. With the slope (m) being 1.7794 and y-intercept (c) being –0.6132, the equation so obtained for the concentration of methanol is: $y = 1.7794x + (-0.6132)$

For the methanol detected samples 2 and 4, the value of y was obtained from the graphs of peak retention time as shown in figure 1 and 2. This was then used to calculate the concentration of the methanol in the detected samples 2 and 4. The concentration of methanol in samples 2 and 4 was 10.050ppm and 13.721ppm respectively.

DISCUSSION

Methanol is cheaper and is a potent adulterant which can lead to hazardous poisoning.[9] Most of these cases of methanol toxicity were reported following the use of traditionally fermented or alcoholic beverages produced illicitly. Instances of methanol poisoning resulting from the consumption of locally produced alcohol have been reported in Nepal in the past.[8]

In 2008 A.D., seven people from Sindhuli and Sindhupalchowk presented to tertiary referral hospital with optical and neurological manifestations following the consumption of locally brewed alcohol.[8] These manifestations were that of methanol toxicity and only two of these seven people had an improvement in their vision.[12] Several cases associated with methanol toxicity have been reported in India. In 2008 A.D., 180 people died in Karnataka and Tamil Nadu with labourers bringing the liquor, and selling it in plastic pouches from illegal shops after mixing it with chemicals. The first case following the consumption of liquor came with symptoms of blurring of vision and vomiting.[13] Similarly, in 2009 A.D., 136 deaths were reported in Ahmedabad, Gujarat, India following the consumption of country-made liquor with the Forensic Science Laboratory reporting a large dose of methyl alcohol.[14] Both these tragedies have been associated with the consumption of local alcohol referred to as Hooch.[13,14]

Further, 25 people died in Indonesia in 2009 following the consumption of fermented palm wine containing methanol.[5] In 2007, 2252 cases of methanol intoxications were reported in the USA.[5]

In 2015 A.D., a study on the Nepalese homebrewed alcoholic beverages was conducted on the basis of its type, ingredients and ethanol concentration using alcohol pycnometer.[15] In Nepal, it was mentioned that home-brewed alcoholic beverages had a higher average concentration of ethanol compared to industrially produced ones.[15] However, no information was provided regarding the methanol content and toxicity of these homemade beverages.[15]

At an elevation of 4679 meters above sea level, winter temperatures in Jumla gets as low as –14°c.[16] At such higher altitude and lower temperatures, the transportation as well as availability of industrial beverages are minimal. In addition to this, the cost for production of local beverages is cheaper and thus leads to higher consumption of these home brewed beverages. And although the endogenous concentration of methanol rarely exceeds 0.15 mg/dl (15ppm) which is unlikely to be toxic, consumption of beverages in massive amounts can lead to accumulation of methanol, leading to the overall increase in the methanol constituent of the body and thus causing toxicity.[17]

The present study showed presence of methanol contamination in two of the four analyzed traditionally fermented alcoholic beverages produced in Chandannath, Jumla. The findings of the present study however contradict with the findings of a similar study conducted by Kaphle et al., in ward number four of Chandannath municipality, Jumla, which did not detect any levels of methanol in the same traditionally fermented home brewed alcoholic beverage that were analyzed in the present study using GC.[11]

The study presents with a limitation of a small sample size in a selected geographic area of Jumla, Nepal. Also not taken into consideration is the methanol vapor which can be produced during the brewing of alcoholic beverages at home which can cause toxicity with the permissible exposure limit of methanol being 200ppm for an 8 hour/day or 40 hour/week exposure.[18] Quantification of vapor form of methanol also becomes essential in further studies due to the risk of exposure to not only the alcohol consuming population but also the inhabitants of the households belonging to the extremes of ages as in the present study.

CONCLUSION

Among four samples of locally brewed alcoholic beverages, methanol was detected in two samples, but at a concentration below the level that would be considered toxic. This finding emphasizes the importance of conducting larger-scale quantification of traditionally fermented alcohol to mitigate the various health risks associated with its potential toxicity, as these beverages are currently not being quantified. The study also paves way for additional study on quantification of traditionally fermented alcoholic beverages for methanol levels on a larger scale. Further, the present study also recommends various concerned authorities to monitor the households where traditionally fermented alcoholic beverages are brewed to minimize a wider public health hazard associated with its consumption as well as exposure to vaporized form of methanol during production.

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