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Quantitative analysis and health risk assessment of methanol in medicinal herbal drinks marketed in Hamadan, Iran

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ABSTRACT

Herbal drinks are one of the most important and widely used pharmaceutical forms of medicinal herbs. Methanol, a neurotoxic agent, occurs naturally at different levels during the production of herbal drinks. The aim of this study was to evaluate contamination and dietary intake of methanol by herbal drinks consumption in Hamadan, Iran. In current study, sixty samples from five types of herbal drinks were analyzed for methanol and ethanol by gas chromatography. The results showed that thirty-three samples were positive for methanol at levels that ranged from 8.35 to 31.90 mg/dl. Additionally, in the positive samples, the ratio of methanol/ ethanol was greater than the European Union acceptable limit. Although methanol intake was estimated within acceptable limits, some of the Iranian consumers might need to moderate their herbal drinks consumption.

INTRODUCTION

The World Health Organization (WHO) has reported that about 80% of the people in developing countries depend on traditional medicine for their health needs (Nili-Ahmadabadi *et al.*, 2011). Herbal extracts, are one of the most important, effective, and widely used pharmaceutical forms of medicinal herbs (Delfan *et al.*, 2014). The Iranian National Standards Institute defines herbal extracts as aromatic distilled waters and saturated aqueous solutions of volatile plant oils or other aromatic substances obtained from fresh or dried plant organs (Namdari *et al.*, 2014). In addition to medical applications, herbal extracts are used as dietary supplements in food industries (Mousavi *et al.*, 2010). In recent years, drinks derived from herbal extracts are commonly used in Iran and other countries. Methanol, a neurotoxic agent (Reddy *et al.*, 2010; Jahan *et al.*, 2015), occurs naturally at different levels during the production of herbal drinks (Mousavi *et al.*, 2010) and alcoholic beverages (Lachenmeier *et al.*, 2008; Croitoru *et al.*, 2013). Methanol toxicity is mediated primarily by formaldehyde and formate, its primary metabolites (Jahan *et al.*, 2015). Formate is metabolized through combination with tetrahydrofolate to produce 10-formyl tetrahydrofolate. This product finally undergoes conversion to water and carbon dioxide (Rachelle and Watterson, 2008). Therefore, the folate storage could be associated with severity of methanol toxicity. The methanol contamination in herbal drinks is a result of demethylation of cell wall pectins by pectin methylesterase, which improves tissue firmness (Mousavi *et al.*, 2010). Previous studies have shown that immersion of thin pieces of vegetable tissue and heating to activate pectin methylesterase results in a rapid increase in methanol in the bathing solution (Anthon and Barrett, 2006).

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Acute methanol toxicity leads to a high-anion-gap metabolic acidosis with an elevated osmolal gap, renal failure, blindness, and even death (Beno *et al.,* 2011). Frequent consumption of some herbal drinks may therefore induce severe damage, particularly blindness (Mousavi *et al.,* 2010). By contrast, chronic methanol toxicity is not well characterized, although recent data have indicated that increases in endogenous levels of formaldehyde due to metabolism of endogenous methanol could be a possible marker for progressive senile dementia (Tong *et al.*, 2011). Shindyapina *et al.* (2014) have also shown that dietary methanol and increases in formaldehyde concentrations in the blood plasma can change the expression of genes involved in the pathogenesis of Alzheimer's disease. Methanol intake due to consumption of herbal drinks could be more important than intake from consumption of alcoholic beverages because the ethanol in alcoholic beverages can antagonize the methanol effects in biological systems. In the present study we aimed to evaluate the intake of methanol by herbal drinks consumption in Hamadan, Iran during the period of October 2014 to March 2015.

METHODS AND MATERIALS

Samples

Sixty samples of five types of commonly-used herbal drinks available in Hamadan, Iran, were purchased from local markets during the period of October 2014 to March 2015. All samples were kept under suitable conditions and were analyzed as soon as possible.

Apparatus and reagents

Methanol and ethanol in the herbal drinks samples were quantitatively analyzed with a gas chromatograph (GC) (Agilent 6850, USA) equipped with a flame ionization detector (FID), a splitless injector, a matrix 80/100 Porapak Q column [0.91 m (L) x 1.8 mm (O.D) x 2 mm (I.D), stainless steel]. Ethanol and methanol with a purity of 99 % (GC grade) were purchased from Merck (Darmstadt, Germany).

Sample preparation

The samples (0.4 mL) were diluted with deionized water into 100 ml volumetric flasks. After 3 min of vortex shaking, 2 µl samples were injected into the GC equipment.

Chromatographic conditions

The carrier gas was nitrogen. The oven temperature was programmed at 50°C for 2 minutes and increased to 140°C for 6 minutes. The injector temperature was set at 160°C, the detector temperature was 220°C; and the gas flow was 30 mL/min. Retention times for methanol and ethanol were 1.7, and 4.4 min, respectively.

Calibration curve

Calibration curves for ethanol and methanol were constructed using external standards in a range from 5 to 50 mg/dl, prepared by dilution of a stock solution in deionized water. The limits of detection (LOD) and limits of quantification (LOQ) were determined as S/N=3 and S/N=9, respectively, where S/N is the ratio of signal/noise in a spiked matrix.

Quality assurance

The reliability of the results of the alcohol analysis was assessed by conducting internal quality control experiments, in addition to using validated methods. In this regard, recoveries of alcohols were recorded by analyzing an herbal drink sample spiked with certain concentrations (5, 15 and 30 mg/dl) of either methanol or ethanol.

Health risk assessment

Daily intake of methanol (DIM) was calculated using the following equation: (DIM) = CM x DI/BW.

In this equation, CM, DI and BW represent the methanol level in herbal drinks (mg/dl), daily intake of herbal drinks and average body weight, respectively. It should be noted that the average of body weight and herbal drinks consumption were assumed 60 kg and 10 ml/day, respectively.

The Health Risk Index (HRI) was determined as the ratio of estimated methanol exposure through consumption of herbal drinks in Iranian population. According to the data in Integrated Risk Information System (IRIS), an oral reference dose (RfD) for methanol is 2 mg/kg/day (US. EPA, 2010). Therefore, The HRI was calculated by the formula DIM/RfD. HRI of more than 1 was considered unsafe for human health.

Statistical analysis

The results were expressed as mean±SD and analyzed using SPSS software version 11.5 through one-way analysis of variance (ANOVA). The $P < 0.05$ was considered statistically significant.

RESULTS

The standard curve data for methanol and ethanol detection using a gas chromatography system are given in Table 1. The r^2 indexes indicated an acceptable calibration curve, which was linear in the 5–50 mg/dl range. Also, the calculated LOD and LOQ values suggested a good performance at low statutory limits. As shown in Table 2, the RSD values of the recovery test were within the acceptable ranges, indicating the good accuracy and precision of this analytical method. In the present study, methanol was detected in 33 (55 %) samples, at levels ranging from 8.35 to 31.90 mg/dl. The mean methanol concentration was 16.92±7.24 mg/dl in contaminated samples. No significant difference was observed in the methanol levels among different herbal drinks. Ethanol was only seen in 8 (13.3%) of the samples, with an overall mean content of 51.63±29.56 mg/dl (Note that ethanol was not detected in herbal distillates except those from *Rosa damascena*). The methanol and ethanol contents in different herbal drinks are presented in Table 3.

Table 1: Linearity range and detection limits for GC-FID analyses.

Alcohols	Range	Linear equation	R^2	LOD (mg/dl)	LOQ (mg/dl)
Methanol	5-50	Y=114.43x + 7.3415	0.9999	0.74	2.46
Ethanol	5-50	Y=146.72x – 1.0966	0.9999	0.52	1.73

Table 2: Recoveries of methanol and ethanol in spiked herbal drinks samples.

Alcohol spiked	Alcohol spiked (mg/dl)	Alcohol found (mg/dl)	Recovery (%)	RSD (%)†
Methanol	5	4.43	88.6	7.8
	15	13.35	89	5.3
	30	28.86	96.2	3.4
Ethanol	5	4.64	92.8	7.7
	15	14.52	96.8	5.8
	30	31.20	104	4.8

†Relative standard deviation (RSD) was obtained from triplicate tests.

Table 3: Methanol and ethanol contents detected in herbal drinks samples.

Herbal distillates	Methanol (mg/dl)				Ethanol (mg/dl)			
	Mean	SD	Max	Min	Mean	SD	Max	Min
Anethum graveolens	17.36	8.22	30.24	9.55	ND†	ND	ND	ND
Cichorium intybus	20.08	9.11	31.90	8.38	ND	ND	ND	ND
Rosa damascena	16.33	6.73	26.94	9.71	51.63	29.56	87.38	9.35
Mentha longifolia	15.23	4.81	21.50	8.39	ND	ND	ND	ND
Salix aegyptiaca	16.03	8.87	31.44	8.35	ND	ND	ND	ND

†Not detected.

Table 4: Daily intake and Health Risk Indexes of methanol through consumption of herbal drinks.

Herbal distillates	Daily intake (mg/kg body weight)			Health Risk Indexes (HRI)		
	Mean	Max	Min	Mean	Max	Min
Anethum graveolens	0.030	0.050	0.016	0.014	0.025	0.008
Cichorium intybus	0.033	0.053	0.014	0.016	0.026	0.007
Rosa damascena	0.027	0.045	0.016	0.013	0.022	0.008
Mentha longifolia	0.025	0.035	0.014	0.012	0.017	0.007
Salix aegyptiaca	0.026	0.052	0.014	0.013	0.026	0.007

DISCUSSION

In this study, occurrence of methanol contamination in herbal drinks was confirmed in Hamadan, Iran. Our findings are comparable to those published in other reports. For instance, Karimi *et al.* (2007) determined that the methanol contamination in 10 different types of herbal drinks ranged from 7.94 to 144.70 mg/dl. A study conducted in Urmia in 2012, which examined six different types of herbal drinks for methanol, detected concentrations that ranged from 7.24 to 27.83 mg/dl (Delirrad *et al.,* 2012). These wide variations in methanol contamination among different studies could be related to the pectin content and pectin methylesterase activity in various plants and also to differences in the processes used in the production of the herbal drinks (Mousavi *et al.,* 2010; Anthon and Barrett, 2006).

The permissible limits for methanol in herbal drinks or other non-alcoholic beverages have not yet been defined, although the European Union (EU) accepts a naturally occurring methanol content of 10 g methanol/L ethanol in alcoholic beverages. This equates to 0.4% (v/v) methanol in an alcoholic drink containing 40% alcohol (Paine and Davan, 2001; Croitoru *et al.,* 2013). Therefore, the acceptable intake of methanol is higher in alcoholic than in non-alcoholic beverages because ethanol is present in alcoholic beverages. In the present study, the ratio of methanol/ ethanol was more than EU acceptable limit. However, even if methanol damage is far less in the presence of ethanol, the chronic use of these products could be considered a health risk.

To evaluate the rate of the risk, DIM and HRI indexes were calculated based on methanol levels in various herbal drinks (Table 4). The results were demonstrated that the methanol level in these products can be considered safe in normal persons if the herbal drink consumption was assumed 10 ml/daily. However, pregnant women, alcoholic patients, and/or elderly persons are especially can be exposed to this risk due to folate deficiency (Croitoru *et al.,* 2013). Thus, these persons as well as people who use herbal drinks more than 375 ml/daily might need to moderate these drinks consumption.

CONCLUSION

Taken collectively, our findings confirmed the occurrence of methanol contamination in more than half of the herbal drinks collected in Hamadan, Iran. The existing evidence indicates that the risk of methanol exposure may be considerable for high risk populations due to consumption of certain kinds of herbal drinks. Therefore, efforts should be taken to increase the quality of these drinks by improving the production processes. Standardization of these products is also recommended.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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