ORIGINAL ARTICLE

Analysis of methanol content in fake alcoholic beverages during a methanol mass poisoning outbreak (Bojnourd- Northeast of Iran, 2018)

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Abstract

Background: Consumption of non-commercial alcoholic drinks is the main cause of methanol poisoning in the world. Non-standardized production methods or deliberate spiking of methanol in non-commercial and homemade alcoholic beverages have been reported, globally. The analysis of toxic alcohol contents in illegally produced alcohol beverages is necessary for prevention and early diagnosis of methanol poisoning especially during alcohol mass poisoning episodes. In this study, we analyzed methanol, ethanol and higher alcohols content in seized illegal alcoholic beverages during methanol mass poisoning outbreak in Bojnourd City (northeast of Iran) in 2018.
Method: During the methanol mass poisoning outbreak in Bojnourd city (northeast of Iran), happened in one –month period from September 22 to October 22, 2018, samples of all illegal alcoholic beverages either found consumed by poisoned patients who admitted to the hospitals or seized by the law enforcement were analyzed. Qualitative and quantitative analysis of ethanol, methanol, 1-propanol, 2-propanol and acetone were performed using GC-FID method.
Results: In the samples collected from a total of 116 seized illegally produced alcohol beverages; methanol was detected in 39.6% with mean concentration of 77.6 %v/v and the range from 12 to 97% v/v. Ethanol was detected in 77 (66.4%) samples with mean concentration of 32.3%v/v. Only 8 (6.9%) samples had a mixture of ethanol and methanol. 1-propanol, 2-propanol and acetone were not detected in any samples.
Conclusion: Methanol is the main toxic chemical component in illegal alcoholic beverages with toxic level and could be considered as a cause of mass poisoning during the alcohol mass poisoning in Bojnourd City. This finding could be considered in planning and implementing of public health measures in Iran.

Keywords: Methanol; Alcoholic beverages; Poisoning; Epidemiology

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INTRODUCTION

Methanol (methyl alcohol, wood alcohol, wood spirit, CH_3OH) is a volatile, flammable, colorless and toxic liquid, produced from distillation of destructed cellulose materials and it may be found in antifreeze products, solvents and engine fuel (1,2). Also, methanol may be detected as a contaminant in alcoholic beverages (3,4).

During the fermentation process for production of the alcoholic beverages, methanol may be produced in low concentrations (3). Methanol contamination in traditionally fermented alcoholic drinks is an increasing problem resulting in poisoning of consumers (3-5). The origins of methanol in fermented alcoholic drinks have not been clearly established. It is likely that the methanol might have been produced due to fermentation of pectin and sugars by microorganisms including yeast, bacteria and fungi containing pectolytic enzymes during the production of non- commercial alcoholic drinks using non-standard and home-made procedures (3-5).

Methanol may also be found in distilled alcoholic beverages as a contaminant especially during illegal and poor quality control production conditions however the source of the methanol in these types of alcoholic drinks is unclear (3,4,6). Methanol has been illegally used in the production of cheap and counterfeit alcoholic beverages. Some profiteers might have deliberately spiked the beverages with methanol or use it instead of ethanol because of its lower price and its availability (7).

Methanol poisoning is one of the main causes of morbidity and mortality as a result of consumption of unrecorded especially counterfeit alcoholic beverages all over the world and it continues to be a serious health concern in many countries (2,8). Methanol converts to toxic metabolites including formaldehyde and formic acid via hepatic alcohol

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dehydrogenase and aldehyde dehydrogenase, respectively. These metabolites cause the toxic presentations such as severe metabolic acidosis, optic neuropathy, cerebral edema and acute renal failure (9). Several major methanol mass poisoning outbreaks in developing countries with high morbidity and mortality have been reported (1,7-10).

Production of the illegal alcoholic drinks for economic benefits remains as a common problem, globally. In addition, in the Islamic countries like Iran, production, distribution, sale and consumption of the alcoholic beverages is prohibited and considered a criminal act according to the legal and religious rules (7,11). Nevertheless, in these countries the production of illegal alcoholic beverages is common (5,7).

Although, the methanol mass poisoning outbreak due to drinking of the illegal and non-standard alcoholic products have been reported, worldwide, there are scant data available about analysis of methanol content in consumed counterfeit alcoholic beverages (7,12).

In this study, we aimed to analyze methanol, ethanol and other toxic alcohols contents in seized unrecorded alcoholic beverages consumed by victims during a methanol mass poisoning outbreak.

METHODS

Sample and data collection

In a cross-sectional study, during the methanol mass poisoning outbreak in Bojnourd city (northeast of Iran), happened in one- month period from September 22 to October 22, 2018, samples of all submitted illegal alcoholic beverages either consumed by poisoned patients who admitted to the hospitals or seized by the law enforcement from the dealers, to the Forensic Toxicology Laboratory of the Legal Medicine Center (Bojnourd -Iran), have been analyzed. Also, the demographic and clinical outcomes of the patients were obtained from hospitals authorities.

Chemicals

Methanol, ethanol, 1-propanol, 2-propanol and acetone were purchased from Merck Co. (Darmstadt, Germany) and used as standards for qualitative and quantitative analysis of the samples. Double distilled water was purchased from Merck- Millipore. All chemicals were of high performance liquid chromatography grade.

Sample preparation

Samples were decanted into a volumetric flask and organoleptic examinations (including: appearance, volume, odor, color, pH (pH meter, Methrom, Switzerland), labeling information (if available) has been applied. For transparent samples, 0.4µL of sample diluted by the double distilled water until final volume reach to 1mL. For turbid samples, the 10mL of sample filtered by Millipore® membrane filter (0.45µm pore size, Merck Co, Germany) and then 2µL of the sample directly injected into GC-FID.

GC- FID Analysis

The samples were analyzed using a Gas Chromatography-Flame Ionization Detector (GC-FID) system (6850 series, Agilent, USA). Separations of methanol, ethanol, 1-propanol, 2-propanol and acetone were accomplished using an Nickel tubing GC packed column (2.0 m, L× 1/8” × 2.0 mm, ID), packed with Porapak Q, 80/100 mesh, pre-conditioned (Agilent, USA). Briefly, the GC-FID parameters were as follows: carrier gas: nitrogen (flow rate: 20 mL/min); Zero air with hydrogen gas as combustion gas; Injector temperature was 150ºC, detector temperature was 300ºC and column temperature was 140ºC. The signals and data acquisition system are processed by a software package (Chem Station®, Agilent, USA). This method has been validated previously and used as a routine analytical method for determining alcohols and related compounds according to the Standard Operating Procedure (SOP) manual in our laboratory.

The quantitative analysis of the target compounds was carried out following calibration of the GC-FID system. The calibration curves were obtained by injections of 1 ml of the standard mixtures containing individual target compounds at concentrations of 0.1,0.2, 0.4, 0.6, 0.8, and 1 volumetric percentage (v/v%) The concentrations of analytes in volume –volume percent (v/v %) were calculated by the data analysis software according to the calibration curves.

Data were analyzing using Statistical Package for the Social Sciences (SPSS) software (SPSS Inc, Chicago) version 20.

RESULTS

During the methanol mass poisoning outbreak, a total of 74 patients were hospitalized over the period of 31 days. Sixty-nine (93.2%) of them were males with a mean age of 31 years (range: 15-61 years old). From the total of 74 patients, 8 were deceased, which made the overall mortality rate of 10.8%. Patients were treated with bicarbonate (94.5%), ethanol (100%), hemodialysis (14.8%), and mechanical ventilation (13.5%) based on their clinical presentations and blood gases. Out of the 66 patients who survived, two patients (3%) had developed toxic optic neuropathy, and one patient (1.5%) blindness. The rest of the survived patients discharged without any long-term complications.

From total of 116 samples that analyzed during the outbreak period, 46 alcoholic beverage samples obtained from poisoned patients and the rest of them were from the alcoholic drinks seized by the law enforcement from the dealers. The organoleptic evaluation results are shown in Table 1. The results showed that most of the samples were packaged in plastic bottles (86.2%), with volume range between 20- 1500 mL. The pH range of samples was 3.6- 4 (44.7%). Most of the samples (67.3%) had clear appearance. Majority of them were odorless (51.5%), followed by ethanol smell (38.6%) (Table 1).

The types of detected alcohols in the samples and their concentrations by v/v % are shown in Table 2. The findings were as followings, methanol was detected in 46 samples (39.6%). Ethanol was detected in 77 (66.4%) samples, 1-propanol, 2-propanol and acetone were not detected in the samples and only 8 (6.9%) samples had a mixture of ethanol and methanol. (Table 2) (Figure 1). Methanol *vs.* ethanol as the only component detected in 38 (32.7%) and 70 (60.3%) samples, respectively. (Figure 2 and 3)

Table 1. Organoleptic characteristics of illegal alcoholic products (n=116)

Organoleptic parameter		Frequency (%)
Type of Packaging	Plastic bottle	86.2
	Glass bottle	13.8
Existence of packaging label	Yes	86.2
	No	13.8
Sample volume (mL)	<50	18.9
	51-100	11.3
	101-200	19.5
	201-300	26.1
	301-400	5.8
	401-500	6.4
	501-1000	7.4
	>1000	4.6
pH	<3	27
	3.1-3.5	None
	3.6- 4	44.7
	4.1- 4.5	None
	4.6- 5	20.1
	5.1- 5.5	None
	5.6- 6	6.0
	>6	2.2
Color	Cherry red	8.5
	Brown	23.1
	Yellow	14.9
	Red	1.6
	Colorless	46.3
	Orange	0.6
	Gray	0.6
	Cream	4.4
Appearance	Clear	67.3
	Opaque	32.7
Odor	Rotten apple	5.8
	Alcohol	38.6
	Vinegar	4.1
	Odorless	51.5

The average concentration of ethanol in the samples was 32.3% v/v, ranging from 2 to 95% v/v mean concentration of methanol in samples was 77.6% v/v ranged from 12 to 97% v/v.

DISCUSSION

Consumption of illegal and non-standard alcoholic drinks is a common cause of methanol mass poisoning around the world (1,2,13-15). In Iran, methanol poisoning continues to be a serious problem and almost all methanol fatalities are related to consumption of illegal and non-commercial alcoholic beverages containing methanol (7,8,16,17). As all activities related to alcoholic drinks are prohibited in Iran, illegally produced and smuggled alcoholic beverages are common and considered as a major health threat. Due to the absence of regulations for legal production of alcoholic beverages, in the context of prohibition of any activities related to them, illegal and homemade production occurs under poor quality conditions. The low price and availability of methanol is considered as significant factors in detecting methanol as main spiked component in illegal and non-commercial alcoholic drinks (7).

One of our important results in the present study was detection of methanol in 36.9% of studied samples, with high and fatal methanol concentrations (77.6% v/v with ranged from 12 to 97% v/v) (5). Also, an interesting finding in this study was detection of methanol as the only alcohol in 38 (32.7%) of all the samples. According to this finding it is concluded that the samples were industrial methanol which distributed and sold as alcoholic beverages. These findings are similar to the study about methanol mass poisoning outbreak with 154 patients in Estonia by Paasma et al. in 2001. During this methanol poisoning episode, some individuals obtained a barrel containing industrial methanol, diluted it with water and sold them in vodka bottles packaging (12). Dadpour et al., showed that the methanol was detected in only less than 0.5% of the seized samples analyzed in Mashhad city (Iran, from March 2013 to March 2014) (18). There has been an obvious rise in the prevalence of methanol contaminated alcoholic beverages in comparison with our study. It should be considered that the methanol concentration exceed 2%v/v in alcoholic beverages would be induced toxicity (5, 19). Also, the European Union limit for naturally produced methanol in alcoholic beverages is 0.4% v/v for methanol at 40% v/v for ethanol (19).

Table 2. Composition of analyzed illegal alcohol samples.

Sample No.	Concentration (%V/V)					Sample No.	Concentration (%V/V)				
	Ethanol	Methanol	1-Propanol	2-Propanol	Acetone		Ethanol	Methanol	1-Propanol	2-Propanol	Acetone
1	6	ND	ND	ND	ND	59	ND	88	ND	ND	ND
2	75	ND	ND	ND	ND	60	ND	88	ND	ND	ND
3	80	ND	ND	ND	ND	61	ND	86	ND	ND	ND
4	90	ND	ND	ND	ND	62	ND	88	ND	ND	ND

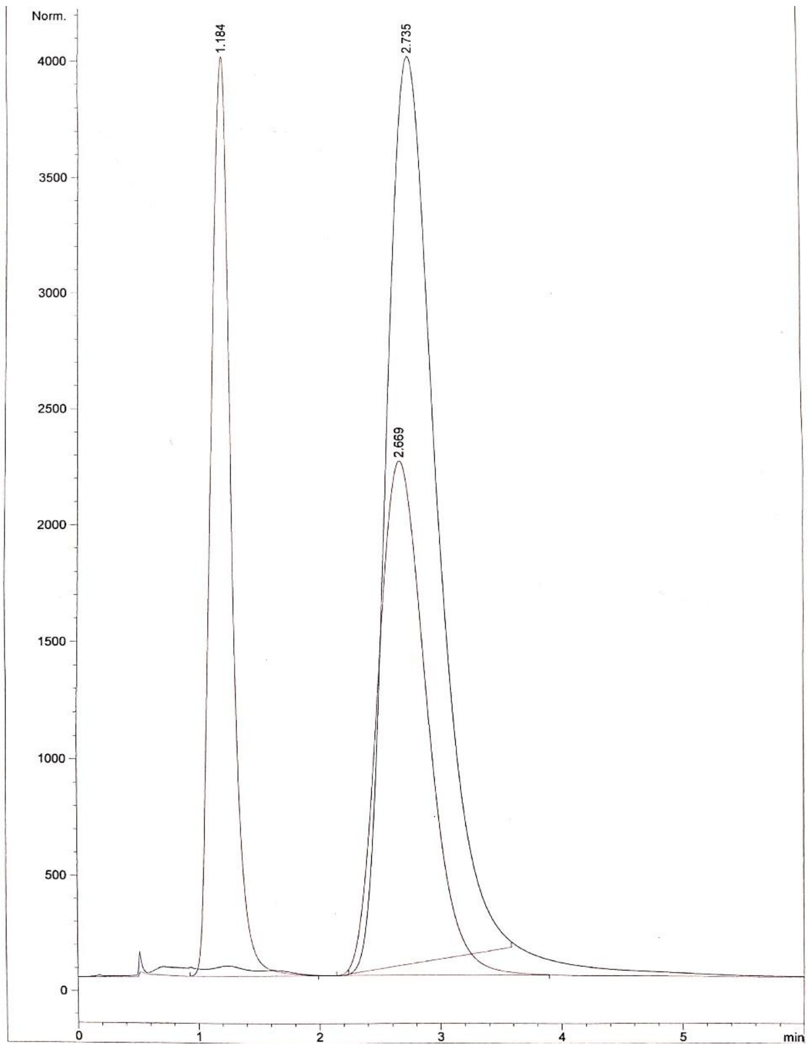
Table 2. Continued.

Sample No.	Concentration (%V/V)					Sample No.	Concentration (%V/V)				
	Ethanol	Methanol	1-Propanol	2-Propanol	Acetone		Ethanol	Methanol	1-Propanol	2-Propanol	Acetone
5	3	ND	ND	ND	ND	63	ND	91	ND	ND	ND
6	ND	45	ND	ND	ND	64	ND	93	ND	ND	ND
7	16	30	ND	ND	ND	65	ND	90	ND	ND	ND
8	80	ND	ND	ND	ND	66	ND	92	ND	ND	ND
9	14	ND	ND	ND	ND	67	ND	93	ND	ND	ND
10	10	ND	ND	ND	ND	68	ND	88	ND	ND	ND
11	9	12	ND	ND	ND	69	ND	97	ND	ND	ND
12	9	15	ND	ND	ND	70	10	ND	ND	ND	ND
13	10	16	ND	ND	ND	71	12	ND	ND	ND	ND
14	7	20	ND	ND	ND	72	10	ND	ND	ND	ND
15	10	32	ND	ND	ND	73	14	ND	ND	ND	ND
16	12	ND	ND	ND	ND	74	12	ND	ND	ND	ND
17	12	ND	ND	ND	ND	75	12	ND	ND	ND	ND
18	37	20	ND	ND	ND	76	12	ND	ND	ND	ND
19	11	33	ND	ND	ND	77	35	ND	ND	ND	ND
20	9	ND	ND	ND	ND	78	7	ND	ND	ND	ND
21	4.5	ND	ND	ND	ND	79	14	ND	ND	ND	ND
22	13	ND	ND	ND	ND	80	7	ND	ND	ND	ND
23	5	ND	ND	ND	ND	81	8	ND	ND	ND	ND
24	8	ND	ND	ND	ND	82	8	ND	ND	ND	ND
25	4	ND	ND	ND	ND	83	11	ND	ND	ND	ND
26	13	ND	ND	ND	ND	84	11	ND	ND	ND	ND
27	7	ND	ND	ND	ND	85	9	ND	ND	ND	ND
28	12	ND	ND	ND	ND	86	12	ND	ND	ND	ND
29	3	ND	ND	ND	ND	87	45	ND	ND	ND	ND
30	ND	88	ND	ND	ND	88	8	ND	ND	ND	ND
31	ND	94	ND	ND	ND	89	33	ND	ND	ND	ND
32	ND	88	ND	ND	ND	90	39	ND	ND	ND	ND
33	ND	86	ND	ND	ND	91	2	ND	ND	ND	ND
34	88	ND	ND	ND	ND	92	41	ND	ND	ND	ND
35	ND	90	ND	ND	ND	93	41	ND	ND	ND	ND
36	91	ND	ND	ND	ND	94	3	ND	ND	ND	ND
37	95	ND	ND	ND	ND	95	8	ND	ND	ND	ND
38	ND	92	ND	ND	ND	96	29	ND	ND	ND	ND
39	ND	92	ND	ND	ND	97	39	ND	ND	ND	ND
40	ND	90	ND	ND	ND	98	3	ND	ND	ND	ND
41	ND	94	ND	ND	ND	99	33	ND	ND	ND	ND
42	ND	90	ND	ND	ND	100	13	ND	ND	ND	ND
43	ND	96	ND	ND	ND	101	41	ND	ND	ND	ND
44	ND	86	ND	ND	ND	102	10	ND	ND	ND	ND
45	ND	86	ND	ND	ND	103	94	ND	ND	ND	ND
46	ND	92	ND	ND	ND	104	89	ND	ND	ND	ND
47	ND	93	ND	ND	ND	105	87	ND	ND	ND	ND

Table 2. Continued.

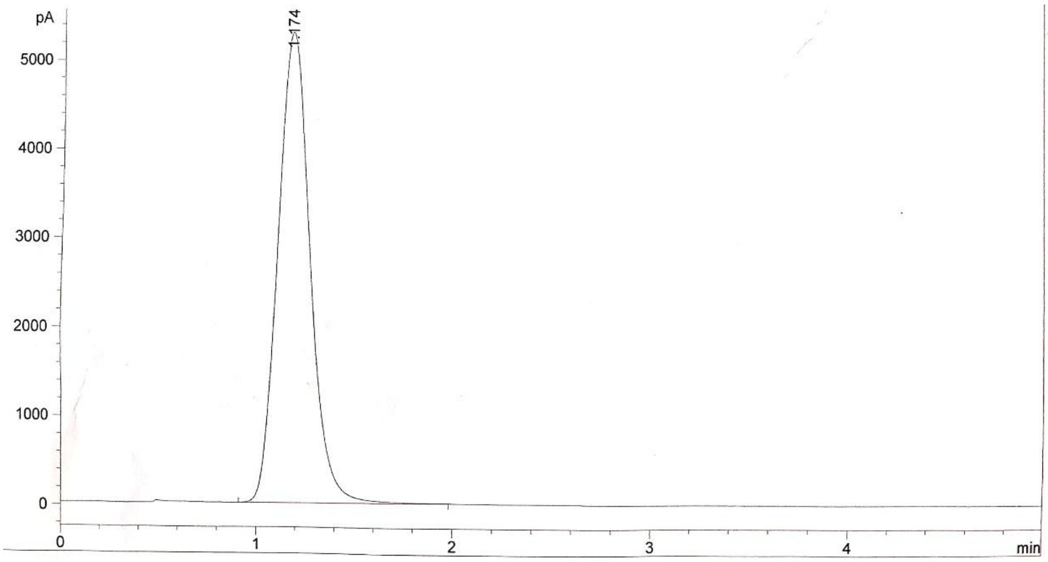
Sample No.	Concentration (%V/V)					Sample No.	Concentration (%V/V)				
	Ethanol	Methanol	1-Propanol	2-Propanol	Acetone		Ethanol	Methanol	1-Propanol	2-Propanol	Acetone
48	ND	92	ND	ND	ND	106	70	ND	ND	ND	ND
49	ND	87	ND	ND	ND	107	83	ND	ND	ND	ND
50	ND	90	ND	ND	ND	108	90	ND	ND	ND	ND
51	ND	93	ND	ND	ND	109	90	ND	ND	ND	ND
52	ND	89	ND	ND	ND	110	75	ND	ND	ND	ND
53	ND	94	ND	ND	ND	111	75	ND	ND	ND	ND
54	ND	94	ND	ND	ND	112	86	ND	ND	ND	ND
55	ND	90	ND	ND	ND	113	86	ND	ND	ND	ND
56	ND	89	ND	ND	ND	114	90	ND	ND	ND	ND
57	ND	92	ND	ND	ND	115	8	ND	ND	ND	ND
58	ND	87	ND	ND	ND	116	18	ND	ND	ND	ND

ND: Not detected



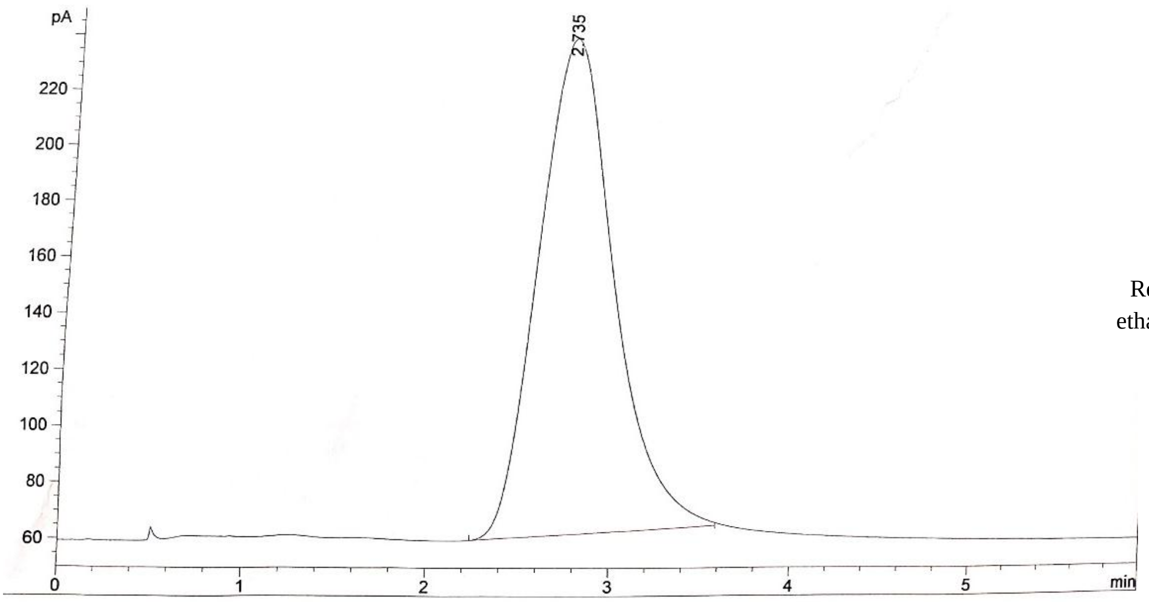
Retention time of methanol: 1.1 minutes
Retention time of ethanol: 2.7 minutes

Figure 1. The chromatogram of the analyzed alcoholic beverages containing methanol and ethanol



Retention time of methanol: 1.1 minutes

Figure 2. The chromatogram of the analyzed alcoholic beverages containing methanol



Retention time of ethanol: 2.7 minutes

Figure 3. The chromatogram of analyzed alcoholic beverages containing ethanol

Our result is different from previous study reported from Turkey by Arslan et al. (2015) in which methanol has been detected in 75% of analyzed illegal alcoholic samples (n=56), however its concentration was in the acceptable level according to Turkish Food Codex (less than 0.4% v/v) (5). In Lao et al (2019) study in Vietnam, 17 samples of total 20 homemade alcoholic drinks made out of rice contained detectable levels of methanol [the median concentration was 9 mg/L (equivalent to 0.001% v/v) (range: 2-37 mg/L) and did not contain sufficient amount of methanol to cause toxicity] (4).

According to the results of these studies, the researchers concluded that the methanol being added to ethanol post production for financial gains (4). In our study, the high concentrations of methanol in the alcoholic drinks analyzed in absence of ethanol and other higher alcohols such as1-propanol and 2-propanol, have been considered as indicator of counterfeit alcoholic beverages. Consumption of these counterfeit/non-commercial/illegally produced alcoholic products could be considered as the main source in methanol mass poisoning.

Previous studies have shown that the illegal and non-standard alcoholic beverages have higher ethanol content compared to the legal products (5,18). For example, Zeren et al. (2012) study from Turkey showed that the ethanol level in illegal alcoholic beverages was detected with an average of 43%v/v (20). Ghadirzadeh et al., in a cross-sectional study on 100 seized alcoholic beverages samples which referred to the laboratory of Legal Medicine Center of Tehran, Iran, during the last 6 months of 2017, showed that the ethanol was found in 95% of samples with a range between 1 - 83% v/v (21). In our study the mean ethanol concentration detected as 32% v/v and it is consistent with other studies findings (5,20, 21). It could be related to similarity in production method for these types of beverages in which distillation as the main method of production.

Alcohols with more than two carbon atoms are defined as higher alcohols (22). Higher alcohols (including 1-propanol, 2-propanol, 1-butanol, 2-butanol, isobutanol, isoamyl alcohol and 1-hexanol) produce naturally in alcoholic beverages as by-products during alcoholic fermentation. The levels of higher alcohols in illicit/unrecorded/homemade produced alcoholic drinks might cause severe health consequences including liver diseases, chronic poisoning and death (23). Lachenmeier *et al.* (2008) proposed a preliminary guideline level would be 1000 g/hl (equivalent to 1.25% v/v) of pure alcohol for the sum of all higher alcohols. In our study, 1-propanol, 2- propanol and acetone were not detected in the samples. It can be explained by the distillation as a main method for production of the non-surrogate (commercial/unrecorded/homemade) alcoholic products.

This study is the first designed research in Iran in which toxic alcohols content in illegal alcoholic beverages during a methanol mass poisoning outbreak has been analyzed. Further studies in other geographical regions in Iran with larger sample sizes are required to provide a clear picture of prevalence, implicating and percolating factors and chemical profiles of non-commercial/counterfeit/ homemade alcoholic drinks should be suggested.

CONCLUSION

Methanol is a main toxic alcohol detected with high concentration in non-commercially produced alcoholic beverages in Iran and it is responsible for induced acute toxicity by consumption of these products. It can be concluded that there is an immediate need for health authorities for massively educating the general population in regard to seriousness of methanol toxicity and non-commercial alcohols containing it. Finally, implementation of strict policies and surveillance of methanol sources control should be proposed as a preventive measure for public access to methanol.

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Conflicts of Interest: None to be declared

Ethical Approval: The ethical committee of the Legal Medicine Research Center approved this project (ID No.20445).

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