Original Article

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Autopsy findings and analytical methods for strychnine detection in fatal cases of strychnine ingestion, referred to Fars Legal Medicine Organization

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Abstract

Strychnine is a toxic alkaloid which might be the source of poisoning with homicidal and suicidal purposes. Eleven fatal cases of strychnine ingestion were considered in this study. Strychnine was isolated from biological samples using the solid phase extraction (SPE) procedure and detected by analytical methods such as thin layer chromatography (TLC), gas chromatography/mass spectrometry (GC-MS) as well as high performance liquid chromatography (HPLC). Eleven cases of strychnine ingestion including 8 men and 3 women with minimum and maximum ages of 17 and 56 years old have been studied. Most of them had an education level of high school and had neither criminal history nor psychological disorders except for 3 cases that were using psychiatric drugs. Facial and ocular congestion, facial cyanosis, lung edema and hemorrhage were found in all of the cases. Hyperemic kidney, brain, liver and meninges and lung collapse were other pathologic findings encountered. Reactive gliosis, subarachnoid focal hemorrhage and 6 cm laceration on the left side of the face were separately found in three cases. Considering highly fatal effects of strychnine and its potential suicidal use, it should be strictly and severely prohibited and watched out for its misuse. On the other hand, the analytical methods used, indicated their reliable, simple, specific and sensible application in forensic and clinical investigations.

Keywords: Fatal ingestion, GC-MS, Solid Phase Extraction, Strychnine.

1. Introduction

Strychnine is a highly toxic alkaloid extracted from Strychnos vomica seeds (1). The poison is rapidly absorbed via ingestion and affects central nervous system (CNS) leading to excitation of all parts of the CNS (2). Due to highly fatal and toxic effects of strychnine it has been used for homicide and suicide attempts recently (3, 4). Early onset of postmortem rigidity and microscopic hemorrhages with minimal degenerative neuronal changes in the spinal cord are caused by strychnine ingestion and it can be detected in the bile and liver (5). Thus, histologic examination and toxicological findings lead to the confirmation of strychnine poisoning (4). In one study, strychnine was detected in liver, kidney, blood, brain, stomach contents, urine, bile, and small and large intestines, using a gas-liquid chromatograph equipped with a flame ionization detector (FID)(6). Other useful analytical methods for determining this toxic agent in biological samples are thin layer chromatography (TLC) (7, 8), high performance liquid chromatography (HPLC) (9, 10) and gas chromatography-mass spectroscopy (GC-MS) (11, 12). In this study we aim to describe some fatal cases of strychnine ingestion isolated by the solid phase extraction (SPE) method and determined using TLC,

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HPLC and GC-MS methods.

2. Material and methods

2.1. Cases' histories

Eleven cases of fatal ingestion of strychnine referred to Fars legal Medicine Organization from 2012 to 2013, were involved in this study.

2.2. Autopsy and samples preparing

To determine cause of the death, all of the mentioned cases were referred to Fars legal medicine organization for autopsy, histopathological examinations and laboratory analytical evaluations. Autopsy findings were noted in special report forms. Specimens were prepared from lung, liver, kidney and brain for histopathologic evaluations and for analytical evaluations stomach contents, blood, urine, bile and liver specimens were analysed.

2.3. Chemicals, equipment and analytical methods

Analytical solvents were of HPLC grade and reagents were of analytical grade, Merck®. Strychnine and Papaverine standards were purchased from the Sigma Chemical Co, St. Louis, MO, USA. Stock solutions were prepared in methanol at the concentration of 1.0 mg/ml. They were diluted to 100 mg/ml to obtain the working solutions and stored at 48˚C. Strychnine was extracted using the solid phase extraction (SPE) method. The general SPE procedure is to load a solution onto the SPE phase, wash away undesired components, and then wash off the desired analytes with another solvent into a collection tube. This separation method uses the same type of stationary phases as the ones used in liquid chromatography columns. The stationary phase is provided in a glass or plastic column above a frit or glass wool. After isolation of agents by SPE method, thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography-mass spectroscopy (GC-MS) were used to detect strychnine in the obtained biological samples.

3. Results and dscussion

Table 1 shows demographic data, autopsy and pathological findings as well as the results of analytical methods used for the detection of the toxic agent and strychnine distribution in the obtained biological samples. Eleven cases of fatal ingestion of strychnine were referred to Fars legal medicine organization from 2012 to 2013 including 8 men and 3 women with the minimum and maximum ages of 17 and 56 years old, died by suicide, homicide or accidental use of or exposure to strychnine. Most of them had education levels of primary and high school, as well as one diploma and two cases of BSc.

Two male cases with the age of 44 and 45, were addicted to opioid and drugs, consuming morphine and methamphetamine. The presence of strychnine was confirmed by GC/MS in 4 cases and TLC as well as HPLC in the other remained cases. One of the tissue samples used for the detection of some of the toxic agents at the legal medicine organization, is the liver in which the toxin is metabolized. In our samples, strychnine was detected in all the liver samples except for two cases that strychnine detection had been confirmed in stomach contents and bile.

In most of the cases, there was no family or clinical information leading to the detection of the cause of death but in some cases white crystalline powder was found beside the victim. There was no specific autopsy and pathognomonic finding, although the pathological findings were parallel to signs of acute poisonings (13, 14). Strychnine poisoning was only confirmed according to the results of the analytical methods performed in the laboratory department of Fars Legal Medicine Organization. Strychnine excites neurons of central nervous system and its excitatory effect leads to hyperreflexia, spasms, convulsions and sever muscular contractions and finally victims die because of respiratory arrest (15-17).

Autopsy and pathologic findings including facial and ocular congestion, facial cyanosis, lung edema and hemorrhage were found in almost all of the cases with different severities. These autopsy and pathological findings are not specific for strychnine intoxication and can be found in other fatal acute poisoning cases(4, 16, 18).

In one of the cases reactive gliosis in the brain as well as chronic hepatitis both detected by histopathological evaluations, were obsereved.

Table 1. Demographic data, autopsy and pathologic and analytical toxicology findings of 11 cases of fatal strychnine ingestion referred to Fars legal Medicine Organization from 2012 to 2013.

Case No.	Gender Marital State	Age	Education	History of Suicide attempt	Criminal History/ Psychological disorders	Opioid or drug abuse	Autopsy and Pathologic Findings	Stomach Content	Visceral organs	Other Specimens
1	Male Single	45	Primary	No	No/No	Yes	Facial and ocular congestion, Hyperemic Brain, Lung Edema and Hemorrhage	GC-MS: Strychnine (Very Strong)	GC-MS: Strychnine, Tramadol	Urine GC-MS: Morphine, Methamphetamine Codeine
2	Male Single	19	High School	No	No/No	No	Facial and ocular congestion and Cyanosis, Lung Edema and congestion, Reactive gliosis in brain, chronic hepatitis	HPLC: Strychnine (Very Strong)	-	Bile HPLC: Strychnine, Salicylic Acid
3	Male Married	44	High School	No	No/ Yes (Psychiatric Drugs)	Yes	Facial and ocular congestion and Cyanosis, Lung Edema and anthracosis, Hyperemic brain	HPLC: Strychnine (Very Strong)	HPLC: Strychnine	Urine TLC: Morphine 2+
4	Female Married	28	BSc	No	No/No	No	Ocular congestion, Subarachnoid focal hemorrhage, Lung anthracosis, collapse, Edema and Hemorrhage	HPLC: Strychnine	HPLC: Strychnine	-
5	Female Single	22	High School	Yes	No/ Yes (Psychiatric Drugs)	No	Facial and ocular congestion and Cyanosis, Pericentral degeneration of the liver tissue, Subarachnoid focal hemorrhage, Lung Edema and Hemorrhage	TLC & HPLC: Strychnine, Maprotiline	-	White Powder HPLC: Strychnine
6	Female Single	27	High School	Yes	No/ Yes (Psychiatric Drugs)	No	Facial and ocular congestion and Cyanosis, Facial 6 cm laceration on the Left Side, Lung Edema, collapse and Hemorrhage	-	TLC & HPLC: Strychnine	White Powder HPLC: Strychnine
7	Male/ Single	17	High School	No	No/No	No	Sever Facial and ocular congestion, Lung Edema and Hemorrhage, Hyperemic Liver and meninges	-	TLC & HPLC: Strychnine	White and crystalline Powder TLC and HPLC: Strychnine
8	Male/ Married	37	High School	No	No/No	No	Sever Facial and ocular congestion, Facial Cyanosis, Lung collapse, Edema and Hemorrhage, Hyperemic Kidney	-	GC-MS: Strychnine, Papaverine	Bile GC-MS: Strychnine
9	Male/ Married	56	Diploma	Yes	No/No	No	Facial and ocular congestion and mild Cyanosis, Lung Edema infarction, Pneumonia, and Hemorrhage	GC-MS: Strychnine	TLC& HPLC: Strychnine, Morphine	-
10	Male/ Married	54	-	No	No/No	No	Facial and ocular congestion and Cyanosis, Lung Edema and Hemorrhage	HPLC, GC-MS: Strychnine	TLC& HPLC: Strychnine	-
11	Male/ Single	25	BSc	No	No/No	No	Facial and ocular congestion and Cyanosis, Lung Edema and Hemorrhage	HPLC: Strychnine	TLC& HPLC: Strychnine, Desipramine	-

Lung anthracosis, lung collapse, hyperemic brain, hyperemic liver and meninges, hyperemic kidney, lung infarction and pneumonia were other pathological finding in our cases.

In a 27 year old female case (Case No 6), a 6 cm transverse laceration was observed on the left side of the frontal part of her face besides abrasion in the upper lip, right side of the nose, cheeck, and left side of the face indicating physical damages before the suicide and death. In her 22 year old sister (Case No 5), pericentral degeneration of the liver tissue and subarachnoid focal hemorrhage were observed. Criminal findings show that these two sisters have been victims of suicide in one period using strychnine that had been available as rodenticide; while white crystalline powder has been also found in the crime scene. It should be noted that these two sisters had previous history of suicide attempts and were consuming psychiatric drugs.

Considering non-specific signs of acute poisoning with fatal poisons such as strychnine, definite detection of the cause of death requires toxicology analytical evaluations. Most reliable analytical methods include TLC, HPLC and GC-MS (19-22) needing good and responsive isolation methods such as solid phase extraction (SPE) (23). In this series study, we used SPE method for the extraction of unknown toxic fatal agent(s). Confirmatory analytical methods used were TLC, HPLC and GC-MS that detected strychnine in stomach contents, visceral organs such as liver and other specimens such as bile, urine and unknown suspicious discovered materials (white crystalline powder was found next to 3 dead cases and were analyzed using TLC and HPLC methods thatresulted in the detection of strychnine intoxication). These analytical methods helped us detect Tramadol, Methamphetamine, Codeine, Salicylic Acid, Desipramine, Papaverine and Maprotiline in obtained biological samples. Since these analytical methods are reliable for the detection of unknown toxic agents, laboratory department of Fars Legal Medicine Organization did not perform all these methods for all obtained specimens and detection of toxin in one sample of each case was enough to report cause of death. However, we performed additional analysis for the detection of other materials such as morphine, methamphetamine and etc.

4. Conclusion

Strychnine is a highly fatal toxic agent that leads to painful morbidity in consumers or exposed individuals. Although its consumption is banned, but it is occasionally diagnosed as cause of death (homicide, suicide or accidental exposure). So, precaution is needed to decrease morbidity of such poisons. This study also emphasizes on the importance of toxicological analysis in detecting acute strychnine poisonings. In fatal cases of strychnine toxicity, cause of death was only determined and confirmed accordcing to the results of the toxicological analytical findings. Used analytical methods indicated their reliable, simple, specific and sensible application in forensic and clinical investigations.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants' relatives included in the study.

Conflict of Interest

None declared.

5. References

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