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Acute Myocardial Infarction Associated with Ingestion of Herbal Mixtures Containing Acetylcholinesterase Inhibitors: A Case Study

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***Abstract*—**We reviewed an unusual case of a 65-year-old male taking an herbal mixture containing compounds with anticholinesterase activity for a long period of time, presented with acute my myocardial infarction and multiple organ dysfunction syndrome followed by death. Clinically, there are findings correlated with anticholinesterase activity, such as bilateral miosis, diaphoresis, vomiting and fasciculation without a history of any toxic ingestion or exposure. Gas chromatography–mass spectrometry screening studies identified the presence of thymol, anethole in the herbal extract and butylated hydroxytoluene in the blood sample. Hence, with this case report, we intend to highlight the necessity of evaluating the long-term use of the herbal mixture.

***Keywords*—**Cholinesterase inhibitors, thymol, anethole, butylated hydroxytoluene, cardiac toxicity and myocardial infarction.

NOMENCLATURE

AChE	acetylcholinesterase
GCS	Glasgow coma scale
Ach	acetylcholine
SPE	solid phase extracted
IBS	irritable bowel syndrome
BHT	butylated hydroxytoluene
ACLS	advanced cardiovascular life support
GCMS	gas chromatography–mass spectrometry

I. INTRODUCTION

MYOCARDIAL infarction is a major global concern [1]. It is caused by ischemia-induced myocardial necrosis. Acetylcholine is a synaptic neurotransmitter that plays a critical role in myocardial function, and it is responsible for the preganglionic parasympathetic neurons innervating the heart. This neurotransmitter is controlled by acetylcholinesterase (AChE), an enzyme that catalyzes the breakdown of acetylcholine (Ach), and other choline esters, a reaction necessary to terminate synaptic transmission. It is essential for the physiological function of the central and peripheral nervous systems. The irreversible inhibition of AChE results in the accumulation of ACh in the synapses, causing nerve firing, thereby causing acute cholinergic manifestations [2], [3]. These manifestations may include bronchorrhea, bronchospasm, miosis, weakness, CNS depression, coma, seizures, prolonged QT interval, ventricular dysrhythmias, and metabolic acidosis [4], [5]. In addition, chronic inhibition of AChE activity, causing failure of nerve impulse conduction and eventual nerve damage [6].

There are varieties of herbal extracts that are reported to inhibit AChE activity. These extracts are becoming popular as remedies for various medical problems. However, there are many adverse effects have been reported with herbal extract usage. Recently, there has been increasing concern about the use of AChE inhibitors [7]. Such results proved the diversity of results depending on the chemical composition. Anethole and thymol have been shown to inhibit AChE activity. Anethole is the active component of anise, whereas thymol is the active component of thyme. Both anise and thyme have been shown to have antispasmodic, antitussive, expectorant, carminative, food flavoring, and preservative properties [8], [9]. However, the major pharmacological properties of thymol and anethole are expectorant and antispasmodic actions [10], [11]. Moreover, butylated hydroxytoluene, which is used as a food antioxidant, has been shown to have anti-AChE activity [12]. The enzyme affinity, combination, cumulative dose, and metabolic processes are important in determining the toxicity of AChE inhibitors [13]. Hence, herbal extracts that are used for medicinal purposes are not considered to be absolutely safe, and several factors need to be considered.

Although several studies have revealed the toxicological effects of herbal ingestion, the potential poisoning resulting from mixed herbs has not been investigated. The present case describes an unusual presentation of a 65-year-old male who ingested an herbal mixture containing AChE inhibitors and subsequently developed acute myocardial infarction and multiple organ dysfunction syndrome followed by death.

II. CASE

A 65-year-old man is brought to hospital with episode of chest pain, shortness of breath, and vomiting. He had irritable bowel syndrome (IBS). The symptoms were not associated with a preceding emotionally or physically stressful event. He had no comorbid illnesses, was a non-smoker, used no illicit drugs, and was not an alcoholic. According to his son, he had

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been taking herbal extracts for more than 10 years for IBS treatment and any influenza attacks. At the emergency department, initial electrocardiography showed an ST segment elevation in the anterior leads (Fig. 1). The patient was intubated and connected to a mechanical ventilator because of a decreased level of consciousness; the Glasgow coma scale (GCS) was 7/15. The patient's vital signs revealed a pulse rate of 100/minute, blood pressure of 121/84 mmHg, a respiratory rate of 17 per minute, an oxygen saturation of 100%, and an afebrile state. He was diaphoretic with upper extremity fasciculation. His cardiovascular examination was normal; chest auscultation revealed bilateral fine basal crepitation. The neurological assessment showed non-reactive pinpoint pupils bilaterally, the plantar reflex was extensor, and deep tendon reflexes were sluggish. A few minutes after endotracheal intubation, the patient developed hypotension with pulseless ventricular tachycardia, so a 200-J biphasic cardioversion shock was administered according to advanced cardiovascular life support (ACLS) protocol as well as cardiopulmonary resuscitation. He revived after 5 minutes and was admitted to the intensive care unit with acute myocardial infarction. The patient received a loading dose of aspirin and clopidogrel and then started on 81 g of aspirin once daily, 75 mg of Clopidogrel once daily, a nitrates patch, 20 mg of Rosuvastatin once daily, and 70 mg of Enoxaparin twice daily.

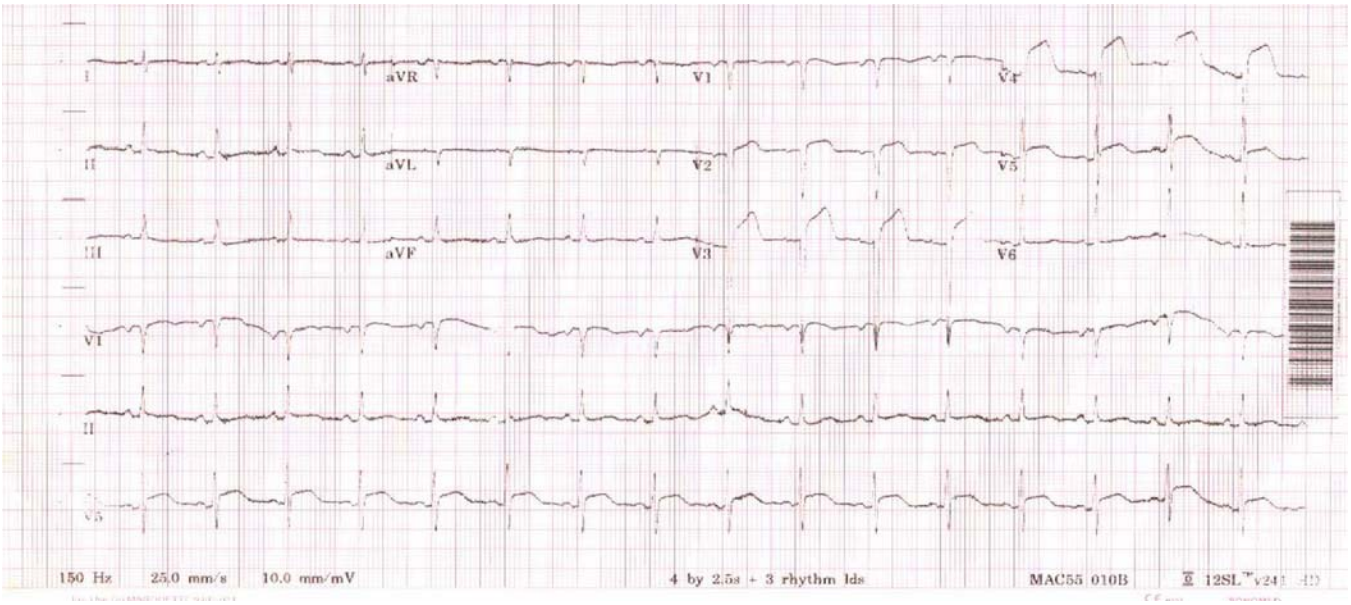

Fig. 1 ECG showing ST segment elevation in V2–V5, suggestive of acute anterior wall MI

The laboratory findings showed a normal complete blood count, normal international normalized ration (1.01), random blood sugar (139 mg/dl), a normal renal and hepatic function profile, and normal serum electrolytes: CK MB: 48.4 ng/ml, {normal value 0–3 ng/ml}, CK 1240 U/L, {normal value 30–135 U/L}. Chest X-ray suggested the presence of acute left ventricular failure, and brain CT scan showed diffuse, mild bilateral hypodensity of the cerebral cortex with poor differentiation of grey/white matter, picture of (global ischemia). Echocardiography demonstrated global hypokinesia with an ejection fraction of 35%.

General unknown screening was conducted using the Abbott Architect ci-4100 system. Based on the patient's toxidromes—miosis, loss of consciousness, and acute myocardial infarction—cholinergic, sedative, hypnotic, and opiate poisoning were suspected. The opiate serum level was zero, the benzodiazepine level was 0.3 ng/ml, barbiturates were less than 25 ng/ml, and the serum cholinesterase level was 7886.4 U/L, all within the normal range. The follow-up sample (3 days after admission) cholinesterase level was 3331.2 U/L, which in comparison to the first result indicated significant serum cholinesterase activity inhibition (>50%). The patient was treated conservatively because it was late for the management of such intoxication with anticholinesterase activity such as atropine and pralidoxime.

We transferred the patient upon his family's request to a higher facility. He was admitted there for four months. The follow-up results showed a hemodynamically stable patient, on a T-piece maintaining an oxygen saturation of 100%, and nasogastric tube feeding.

The laboratory data are shown in Table I. The patient expired after four months due to disseminated intravascular coagulopathy and multiple organ dysfunction syndrome.

III. Materials and Methods for GCMS Experiment

A. Sample Pretreatment

Serum samples were extracted by a solid phase extracted (SPE) method according to the SPE manufacturer's protocol by using a CLEAN SCREEN® DAU SPE cartridge. A total of 1 ml of serum sample was diluted with 2 ml of phosphate buffer (pH 6) prior to SPE. The SPE cartridge was conditioned with 3 ml of methanol, 3 ml of de-ionized water (DI), and 1 ml of phosphate buffer (pH6). Then, the sample was loaded at 1

ml/min followed by washing steps with 3 ml of DI, 1 ml of 0.1M acetic acid, 5 minutes of drying, and 0.2 ml of n-hexane. Acidic and neutral analytes were first eluted by 3 ml of the first elution mixture (Ethyl-acetate 50% – n-Hexane 50%). Then, the cartridge was again washed with 3 ml of methanol and dried for 5 minutes before eluting the basic analytes with 3 ml of the second elution mixture (Dichloromethane 78% – Isoproanol 20% – Ammonia 2%). Both elutions were combined in one tube and evaporated to dryness under a nitrogen stream. Finally, the residues were reconstituted in 100 µl of methanol and made ready for GCMS analysis.

TABLE I
LABORATORY DATA AT TIME OF ADMISSION AND THREE MONTHS LATER

Component	Normal value	At admission	Three months later
White blood cells	4–11 x 10^9/L	11.4	17.4
Hemoglobin	13–17 g/dL	9.1	7.0
Platelets	150–400 x 10^9/L	105	70
International normalization ratio	0.9–1.2	1.7	2.2
Serum creatinine	0.8–1.3 mg/dL	0.5	0.9
Alanine aminotransferase	5–30 U/L	73	75
Aspartate aminotransferase	5–30 U/L	85	71
Serum albumin	3.5–5 g/dL	2.7	2.9
Total bilirubin	0.1–1.2 mg/dL	4.0	5.4
Direct bilirubin	0.1–0.4 mg/dL	2.7	4.1
Alkaline phosphatase	45–115 U/L	661	793
Gamma glutamyl transferase	9–48 U/L	222	256
Others: high D-dimer (1390 ng/ml), normal serum amylase and lipase, negative hepatitis B and hepatitis C virus serology, negative HIV screen.			

TABLE II
THE MAJOR CHEMICAL COMPOUNDS IDENTIFIED IN BLOOD AND HERBAL MIXTURE EXTRACTS BY GC-MS ANALYSIS.

S. N.	Sample	Compound name	Retention time	Molecular formula	m/z fragments	Area %
1	Blood	Butylated hydroxytoluene (BHT)	7.81	$C_{15}H_{24}O$	205,57,220,206	10.0
2	Herbal mix	Thymol	5.98	$C_{10}H_{14}O$	135,150,91,115	10.96
3	Herbal mix	Anethol	6.02	$C_{10}H_{12}O$	148,147,117,77	2.26
4	Herbal mix	Catechol	5.54	$C_6H_6O_6$	110,64,63,81	16

For the herbal mix, approximately 5 grams were ground using a marble mortar and mixed with 20 ml of methanol in a 50-ml vortex tube and vortexed for 2 minutes. A total of 50 µl of the methanolic extract were diluted in 950 µl of methanol and made ready for GCMS analysis.

B. GCMS Analysis

The GCMS analysis was performed using a general screening method in Agilent Tech [14]. The separation column was from Thermo Scientific (TR-5MS) and had the following properties: 30 m length, ID 0.25 mm, and film thickness 0.25 µm. The carrier gas was helium, and the flow rate was 1 ml/min. A total of 2 µl of each sample was injected in a splitless mode at an injection port with a temperature of 260 ºC. The GC thermal program started at 80 ºC and was held for 1.5 min and then increased at the first ramp to 210 ºC at a rate of 30 ºC/min, and then the rate was slowed to 20 ºC/min to reach the final temperature of 320 ºC and held at this temperature for 11 minutes. The ion source in MS was electron ionization (EI), and the analysis was conducted in a scanning mode with electron energy of 70 eV. The ion source and transfer line temperatures were adjusted to 230 ºC.

IV. RESULTS AND DISCUSSION

In the present case study, unknown screening method was used and blood and herbal samples were extracted and examined by gas chromatography–mass spectrometry (GCMS, Agilent tech.) using the general screening method. The identification of chemical constituents present in the blood and herbal extract was confirmed based on the matching between the acquired mass spectra, molecular formula, and molecular weight and reference standard spectra of the NIST's library database. Similarity above 90% was considered as sufficient matching for compound identification. All identified compounds in this study had similarities above 90% when searched in the NIST standard database available in the GCMS system. Fig. 2 illustrates that the total ion chromatograph of the blood sample, acquired mass spectra, and database spectra of the BHT appeared almost identical. The GCMS analysis of the blood and herbal extract revealed the presence of chemical compounds that could contribute to the toxicity syndrome appearing in the patient. The major active components identified were thymol, anethole, and BHT (Table II). The other identified peaks included 1,4-diethenyl Benzene, p-Acetonylanisole, 4-tert-Butylcatechol, Catechol, 3,4-Dihydroxytoluene, 3-Butenyl isothiocyanate, 2,4-di-t-Butylphenol and gamma-Tocopherol. Other unmentioned peaks may have resulted from reactions of the co-extracted products.

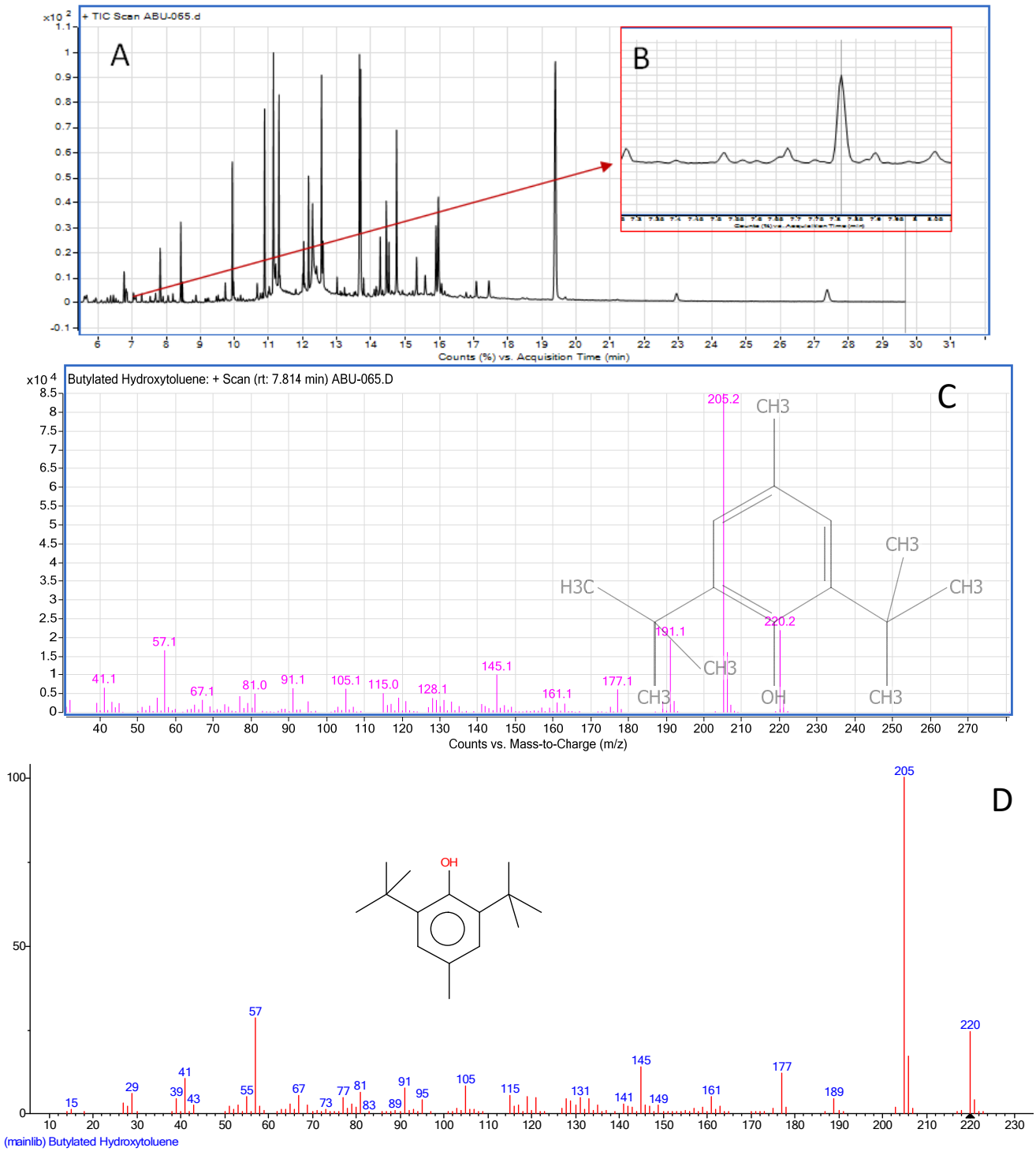

Fig. 2 A) Total Ion Chromatogram (TIC) of serum sample B) Zoom in of the TIC to demonstrate the BHT peak at RT 7.814 C) Acquired spectra from the serum sample D) Standard reference spectra of the NIST library database

Anethole and thymol are the major components of anise and thyme respectively [15], [16]. They exhibit multiple pharmacological actions and have been shown to have anticholinesterase activity [17], [18]. BHT is widely used as a food antioxidant and has previously been shown to decrease cholinesterase activity and to exhibit harmful effects on the blood [12], [19]. Although thymol, anethole, and BHT have been shown to inhibit cholinesterase enzyme, the consumption of the normal amount found in food is not thought to represent a risk to human health.

As the herbal extract was consumed in large quantities for medicinal purposes, the cholinergic syndrome observed in this patient may be attributed to the anticholinesterase activity of thymol and anethole. In addition, the delayed cholinesterase

inhibition may be attributed to the lipophilicity of thymol and anethole, which were thus distributed into fat tissues, and the slow conversion of these compounds may have delayed the toxic effects [20], [21]. The presence of BHT in the blood sample but not in the herbal extract may be attributable to the metabolic pathway of the herbal constituents, such as 2-Hydroxy-4-methylphenol (4-Dihydroxytoluene) and 2,4-di-t-Butylphenol, that are used in BHT synthesis [22].

The usage of herbal supplements for treating health problems has increased and become a major global concern due to the lack information about chemical interactions and the toxic effects of herbal combinations. Exposure to herbal mixtures may be synergistic or additive, thus leading to toxic effects exceeding those noted for exposure to a single component [23]. For example, pesticide combinations have been shown to be lethal at concentrations that were sublethal for single components due to the additive AchE inhibition [24]. Moreover, while low doses of a single component may not cause toxic effects, when combined with other components, they may result in increased toxic effects. Interestingly, experiments in non-human primates have shown that combinations of thymol and anethole exhibit a synergistic effect on enzyme inhibition and mortality [25], [26]. Therefore, in this case, the consumption of large quantities of herbal products containing lipophilic anticholinesterase-active substances may be a reasonable explanation for this delayed cholinesterase enzyme inhibition. With regard to cardiovascular toxicity, there is evidence that anethole and thymol toxicity is mediated through AChE inhibition with a subsequent increase in the level of Ach in the myocardium [27].

Cardiac abnormalities such as sinus bradycardia, prolonged PR interval, sinus tachycardia, prolonged corrected QT interval, and ventricular arrhythmia have been reported in cases of intoxication by ChE inhibitors [27]. However, these abnormalities have rarely been reported in cases of intoxication by herbal extracts containing ChE inhibitors such as thymol and anethole.

The cardiovascular toxicity of anethole and thymol has been reported. A study was conducted in isolated rat aortas showing that lower concentrations of anethole result in the induction of contractility, whereas the relaxant effects were observed at higher concentrations [28]. In other experiments, anethole has been associated with dose-dependent hypotension and bradycardia followed by a significant pressure effect associated with delayed bradycardia. The hypotension and bradycardia response of anethole seems to be caused by a cholinergic mechanism of anethole, most likely through inhibition of AChE [29]. Conversely, it has been demonstrated that thymol can induce cardiac toxicity, producing hypotension and suppressed cardiac ionic channels in a concentration-dependent manner [30], [31]. In addition, cardiac arrest has been reported as a toxic effect of thymol, albeit a rare one [32].

Although we found no anethole and thymol in the blood sample that might have been due to a fast metabolism and high excretion rate, the BHT in the blood sample is another suspected compound behind the cardiac toxicity in the current case. BHT poisoning has rarely been reported in the literature, and there is, to the best of our knowledge, no single report of BHT-induced cardiac toxicity to date. However, *in vitro* and *in vivo* studies have previously shown cardiac effects of BHT, such as inhibitory actions in atrial contractions [33], altered total lipid content in the heart [34], and injuries to myocardial cells [35]. Multiple organ failures and fatalities have been reported in humans exposed to BHT-containing products [36], [37].

In conclusion, based on the results obtained from the GCMS screening studies, the presence of thymol and anethole was identified in the herbal sample, whereas BHT was identified in the blood sample. Therefore, we consider AChE inhibitor intoxication in the presence of the cholinergic toxidrome following the exposure to herbal mixtures containing AChE inhibitors. These compounds may be responsible for this rare case of intoxication and acute myocardial infarction. Dyselectromedia and hypoxemia may be the contributing factors. Thus, along with initial supportive treatment, intensive cardiac monitoring should be done to reduce mortality in such cases.

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Conflict of interest: The author declares that they have no conflict of interest.

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REFERENCES

[1] Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, et al. Heart disease and stroke statistics--2013 update: A report from the American Heart Association. Circulation. 2013;127(1):e6-e245. Epub 2012/12/15.

[2] Costa LG. Current issues in organophosphate toxicology. Clinica chimica acta; international journal of clinical chemistry. 2006;366(1-2):1-13. Epub 2005/12/13.

[3] Vale A, Lotti M. Organophosphorus and carbamate insecticide poisoning. Handbook of clinical neurology. 2015;131:149-68. Epub 2015/11/14.

[4] Siegal D, Kotowycz MA, Methot M, Baranchuk A. Complete heart block following intentional carbamate ingestion. The Canadian journal of cardiology. 2009;25(8):e288-90. Epub 2009/08/12.

[5] Sekijima Y, Morita H, Shindo M, Okudera H, Shibata T. (A case of severe sarin poisoning in the sarin attack in Matsumoto--one-year follow-up of clinical findings, and laboratory data). Rinsho shinkeigaku = Clinical neurology. 1995;35(11):1241-5. Epub 1995/11/01.

[6] Yanagisawa N, Morita H, Nakajima T. Sarin experiences in Japan: acute toxicity and long-term effects. Journal of the neurological sciences. 2006;249(1):76-85. Epub 2006/09/12.

[7] Afshari AR, Sadeghnia HR, Mollazadeh H. A Review on Potential Mechanisms of *Terminalia chebula* in Alzheimer's Disease. Advances in pharmacological sciences. 2016;2016:8964849. Epub 2016/03/05.

[8] Barnes J, Anderson LA, Phillipson JD. Herbal Medicines: Pharmaceutical Press; 2007.

[9] Chizzola R, Michitsch H, Franz C. Antioxidative properties of *Thymus vulgaris* leaves: Comparison of different extracts and essential oil chemotypes. Journal of agricultural and food chemistry. 2008; 56(16):6897-904. Epub 2008/07/29.

[10] Hosseinzadeh S, Kukhdan AJ, Hosseini A, Armand R. The Application of *Thymus vulgaris* in Traditional and Modern Medicine: A Review. Global Journal of Pharmacology. 2015;9(3):260-6.

[11] Idle JR. Christmas gingerbread (Lebkuchen) and Christmas cheer--review of the potential role of mood elevating amphetamine-like compounds formed *in vivo* and *in furno*. Prague medical report. 2005;106(1):27-38. Epub 2005/07/13.

[12] Choe SYY, K.H. Toxicological studies of antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Korea Advanced Inst of Science and Technology. 1982;14(3).

[13] Cedergreen N. Quantifying synergy: A systematic review of mixture toxicity studies within environmental toxicology. PloS one. 2014;9(5):e96580.

[14] Hassan AA, Abdullah SM, Khardali IA, Shaikhain GA, Oraiby ME. Evaluation of pesticides multiresidue contamination of khat leaves from Jazan region, Kingdome Saudi Arabia, using solid-phase extraction – gas chromatography/mass spectrometry. Toxicological & Environmental Chemistry. 2013;95(9):1477-83.

[15] Orhan I, Kartal M, Kan Y, Sener B. Activity of essential oils and individual components against acetyl- and butyryl-cholinesterase. Zeitschrift fur Naturforschung C, Journal of biosciences. 2008;63(7-8):547-53. Epub 2008/09/25.

[16] Hudaib M, Speroni E, Di Pietra AM, Cavrini V. GC/MS evaluation of thyme (*Thymus vulgaris* L.) oil composition and variations during the vegetative cycle. Journal of pharmaceutical and biomedical analysis. 2002;29(4):691-700. Epub 2002/07/03.

[17] Rodrigues VM, Rosa PT, Marques MO, Petenate AJ, Meireles MA. Supercritical extraction of essential oil from aniseed (*Pimpinella anisum* L) using CO2: solubility, kinetics, and composition data. Journal of agricultural and food chemistry. 2003;51(6):1518-23. Epub 2003/03/06.

[18] Bhadra S, Mukherjee PK, Kumar NS, Bandyopadhyay A. Anticholinesterase activity of standardized extract of *Illicium verum* Hook. f. fruits. Fitoterapia. 2011;82(3):342-6. Epub 2010/11/16.

[19] Jayalakshmi CP, Sharma JD. Effect of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on rat erythrocytes. Environmental research. 1986;41(1):235-8. Epub 1986/10/01.

[20] Jukic M, Politeo O, Maksimovic M, Milos M. In vitro acetylcholinesterase inhibitory properties of thymol, carvacrol and their derivatives thymoquinone and thymohydroquinone. Phytotherapy research: PTR. 2007;21(3):259-61. Epub 2006/12/23.

[21] Reiner GN, Labuckas DO, Garcia DA. Lipophilicity of some GABAergic phenols and related compounds determined by HPLC and partition coefficients in different systems. Journal of pharmaceutical and biomedical analysis. 2009;49(3):686-91. Epub 2009/02/04.

[22] Mcconnell WV, Davis HE. Alkylation of phenols. Google Patents; 1963.

[23] Segvic Klaric M, Kosalec I, Mastelic J, Pieckova E, Pepeljnak S. Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. Letters in applied microbiology. 2007;44(1):36-42. Epub 2007/01/11.

[24] Iten F, Saller R, Abel G, Reichling J. Additive antimicrobial (corrected) effects of the active components of the essential oil of *Thymus vulgaris*--chemotype carvacrol. Planta medica. 2009;75(11):1231-6. Epub 2009/04/07.

[25] Laetz CA, Baldwin DH, Collier TK, Hebert V, Stark JD, Scholz NL. The synergistic toxicity of pesticide mixtures: Implications for risk assessment and the conservation of endangered Pacific salmon. Environmental health perspectives. 2009;117(3):348-53. Epub 2009/04/02.

[26] Pavela R. Acute toxicity and synergistic and antagonistic effects of the aromatic compounds of some essential oils against *Culex quinquefasciatus* Say larvae. Parasitology research. 2015;114(10):3835-53. Epub 2015/07/08.

[27] Koul O, Singh R, Kaur B, Kanda D. Comparative study on the behavioral response and acute toxicity of some essential oil compounds and their binary mixtures to larvae of *Helicoverpa armigera*, *Spodoptera litura* and *Chilo partellus*. Industrial Crops and Products. 2013;49:428-36.

[28] Karasu-Minareci E, Gunay N, Minareci K, Sadan G, Ozbey G. What may be happen after an organophosphate exposure: Acute myocardial infarction? Journal of forensic and legal medicine. 2012;19(2):94-6. Epub 2012/01/28.

[29] Soares PM, Lima RF, de Freitas Pires A, Souza EP, Assreuy AM, Criddle DN. Effects of anethole and structural analogues on the contractility of rat isolated aorta: Involvement of voltage-dependent Ca2+-channels. Life sciences. 2007;81(13):1085-93. Epub 2007/09/18.

[30] de Siqueira RJ, Magalhaes PJ, Leal-Cardoso JH, Duarte GP, Lahlou S. Cardiovascular effects of the essential oil of *Croton zehntneri* leaves and its main constituents, anethole and estragole, in normotensive conscious rats. Life sciences. 2006;78(20):2365-72. Epub 2005/12/06.

[31] Szentandrassy N, Szigeti G, Szegedi C, Sarkozi S, Magyar J, Banyasz T, et al. Effect of thymol on calcium handling in mammalian ventricular myocardium. Life sciences. 2004;74(7):909-21. Epub 2003/12/09.

[32] Magyar J, Szentandrassy N, Banyasz T, Fulop L, Varro A, Nanasi PP. Effects of thymol on calcium and potassium currents in canine and human ventricular cardiomyocytes. British journal of pharmacology. 2002;136(2):330-8. Epub 2002/05/16.

[33] Gad SC, Leslie SW, Acosta D. Inhibitory actions of butylated hydroxytoluene on isolated ileal, atrial, and perfused heart preparations. Toxicology and applied pharmacology. 1979;49(1):45-52. Epub 1979/06/15.

[34] Saheb JL, Saheb SA. Toxic effects of butylated hydroxytoluene on rats. Canadian journal of comparative medicine: Revue canadienne de medecine comparee. 1977;41(2):195-201. Epub 1977/04/01.

[35] Leslie SW, Gad SC, Acosta D. Cytotoxicity of butylated hydroxytoluene and butylated hydroxyanisole in cultured heart cells. Toxicology. 1978;10(3):281-9. Epub 1978/07/01.

[36] Lin CH, Yang JY. Chemical burn with cresol intoxication and multiple organ failure. Burns: journal of the international society for burn injuries. 1992;18(2):162-6. Epub 1992/04/01.

[37] Monma-Ohtaki J, Maeno Y, Nagao M, Iwasa M, Koyama H, Isobe I, et al. An autopsy case of poisoning by massive absorption of cresol a short time before death. Forensic science international. 2002;126(1):77-81. Epub 2002/04/17.