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Spasmolytic Effect of Black Bile-reducing Plants from Lamiaceae: the correlation between Traditional Iranian Medicine and Pharmacological and Phytochemical surveys

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Abstract

The roots of Traditional Iranian Medicine (TIM) go back to thousand years ago. Based on TIM, black bile, one of the four humors within the body, is the concentrated part of the blood representing a cold and dry quality. Black bile tends to deposit in tissues, leading to diseases such as spasm, which is a painful paralysis-like immobility condition. One of its possible causes is muscle dehydration, resembling the dryness caused by dominance of black bile. In TIM, several medicinal plants are claimed to be effective in the regulation of black bile; among them, the presence of the Lamiceae family is very notable. In this review, the relationship between spasm as one of the symptoms of increasing black bile in the body was discussed. Also, the compounds reported in the black-bile eliminating plants have been found in the literature. The majority of them were from monoterpenes and sesquiterpenes such as citral, carvacrol, fenchone and pulegone in the essential oil of black bile reducing plants. The main compounds properties of black bile reducing plants can be used to orient quantitative system pharmacology models in further studies.

Keywords: Black bile, Spasm, Lamiaceae, Traditional Iranian Medicine, Essential oil.

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1. Introduction

According to Traditional Iranian Medicine (TIM), black bile (Soda) is one of the four humors within the human body; human health is related to the equilibrium quantity and quality of these humours. Black bile was believed that is produced in liver, while its storage and regulation is in the spleen (1). It is cold, dry, and sparse in the body. Natural black bile is the concentrated part of the blood and essential for the proper function of organs; it keeps bones, teeth, and tendons healthy and strong. However, abnormal black bile is the result of combustion and burning of humors (2). Due to the characteristics and properties of abnormal black bile, it tends to deposit in any tissue of the body, which eventually causes disease. For instance, the accumulation of black bile in the skin, blood, stomach, and brain causes dark spots, thick blood, false appetite, and melancholia, respectively. Gastric spasms, laryngospasm, and muscle cramps, which occur due to muscle spasms, are symptoms of built up excessive or abnormal black bile in the body (3).

A muscle cramp is defined as an involun-

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tary contraction of a group of muscles that leads to paralysis-like immobility with mild-to-severe pain (4). The main cause of spasm is not exactly known and several factors are involved; however, muscle spasms can be divided into three groups: (a) Due to some diseases and pathological conditions (including diabetes, neuropathy, and metabolic disorders.); (b) idiopathic nocturnal spasm that occurs at night during sleep with no specific cause; (c): Exercise-associated muscle cramps (EAMC) occurring due to exercise. There are two hypotheses about the causes of exercise-induced spasms: the dehydration or electrolyte depletion hypothesis, and the neuromuscular hypothesis. According to the dehydration hypothesis, spasm is caused by excessive sweating and not replacing enough fluids (5). Muscle contraction begins with the motor nerve's stimulation and once the action potential reaches the axon terminal, acetylcholine is released. Then, the binding of acetylcholine to the receptors in the muscle cell membrane leads to the activation of fast sodium channels in the muscle cell membrane; this process is called depolarization. In addition, the action potential causes the release of a large bolus of Ca^{2+} from the sarcoplasmic reticulum (SR) into the sarcoplasm by stimulating the dihydropyridine receptor (DHPR) of the T-tubules which is a voltage-gated Ca^{2+} channel (6).

The Lamiaceae (Labiatae) family belongs to the group of flowering plants and includes about 236 genera and 6900-7200 species (7). Some of the most famous members of this family include thyme, mint, oregano, basil, rosemary, hyssop, and lemon balm (8). There is a wide range of compounds such as terpenoids, iridoids, small phenolics, and flavonoids in the Lamiaceae family. The smell and taste of these plants are due to the presence of some of the short-chain terpenoids (9). There are 46 genera and 410 species and subspecies of the Lamiaceae family in Iran, while 124 species and subspecies (30%) are endemic to Iran (10)

Today, the trend toward traditional, complementary, and alternative medicine and making more use of nature is a global concern. Since 2002, the World Health Organization (WHO) has recommended the use of these human sciences by providing a definition of traditional and complementary medicine (11). TIM has several thousand years of history and contains the experiences of our ancestors. In an area where databases have expanded dramatically and the human needs to discover new treatment methods is still significant, it is necessary to take advantage of the potential of traditional medicine and establish a connection between it and modern medicine. The aim of this study was to interpret spasm as one of the symptoms of black bile by using today's knowledge and also to introduce the molecular and cellular mechanisms of the antispasmodic effect of Lamiaceae family as the common black bile-reducer plants in the TIM documents. It is worth mentioning that integration and interpretation of data related to the biological activities of medicinal plants and their use in traditional medicine may be effective in clarifying their mechanism of action.

2. Material and methods

In this paper, herbal treatments against black bile were reviewed in a TIM manuscript, “Makhzan al advia” (12). There are several plants proposed in TIM for the management and reduction of black bile. The present study was conducted to review these plants to find their phytochemical constituents and the evidence for their efficacy to understand the biological mechanisms of selected plants in modern medicine. In order to achieve this aim, electronic databases including PubMed, Scopus, Science Direct, and Google Scholar were searched for these plants plus keywords including pharmacological effect, essential oil, review, phytochemical, secondary metabolites, spasm, antispasmodic, and spasmolytic between 2010 and 2022.

3. Result

There are some medicinal herbs which were used in TIM for the management and removal of black bile from body. *Lavandula stoechas* L., *Mentha pulegium* L., *Origanum majorana* L. and *Teucrium chamaedrys* L. were prepared as a decoction in TIM. However; the exact method of preparation for *Ocimum basilicum* L., *Dracocephalum moldavica*, L., *Melissa officinalis* L. and *Thymus vulgaris* L. were not mentioned in the

literature. Based on the result, there are scientific evidence for antispasmodic effect of these plants, which belong to the Lamiaceae family.

3.1. Dracocephalum moldavica L.

There were not enough studies to confirm the antispasmodic effect of *Dracocephalum moldavica* L.

3.2. Lavandula spp

In a clinical trial that was conducted on 80 patients undergoing angiography, no significant difference was observed between the heart rate, SBP and DBP of the patients who inhaled lavender oil at the night before the angiography and the group that received oxazepam, and in both groups all parameters were significantly reduced at the end of the study compared to the baseline (13). In a case report, prescribing a poly herbal decoction including *Lavandula stoechas* for a 21-year-old man suffering from asthma for ten days improved his symptoms and reduced the need to use inhalers that was claimed to be due to the relaxing effect of *L. stoechas* (14). According to studies, *L. angustifolia* has the ability to inhibit spasm in the guinea pig ileum and rat uterus through postsynaptic action and cAMP (15). In another study, a dose-dependent antispasmodic effect on spontaneous contractions in isolated rabbit jejunum was observed from the aqueous methanolic extract of *L. stoechas* through blocking calcium channels (16).

3.3. Melissa officinalis L.

The effect of *Melissa officinalis* in reducing the spasmodic pains of dysmenorrhea has been proved in a double-blind clinical trial that included 110 girls who received three capsules containing 330 mg of *M. officinalis* daily for three days at the beginning of hemorrhage (17). In a meta-analysis of clinical trials, the effectiveness of the herbal formula known as STW-5, which contains *M. officinalis*, on the spasm-related dyspepsia has been proven (18). Also, in a randomized single-blind clinical trial, it has been found that *M. officinalis* compared to mefenamic acid, was more effective in reducing after-pain symptoms (19). In another clinical trial, the effect of *M. officinalis* on the treatment of bruxism has been investigated and it has been determined that the use of this plant had no significant effect on bruxism compared to placebo (20).

In a study conducted to determine the antispasmodic effect of *M. officinalis* on isolated rat intestine, the extract prepared by maceration with a concentration of 4 mg/ml had a spasmolytic effect similar to that of 7-10 molar verapamil; the proposed mechanism for this effect is the inhibition of muscarinic receptors and calcium channels (21). The findings of an animal study, indicated that *M. officinalis* aqueous extract was able to relax isolated endothelium-intact aortic rings in STZ-diabetic rats through the nitric oxide pathway (22).

3.4. Mentha pulegium L.

Based on the study conducted on *M. pulegium*, the different extracts of this plant were able to relax the isolated rat ileum; however, the dichloromethane fraction had the strongest antispasmodic effect through blocking the calcium channels (15). In another study, the essential oil of *M. pulegium* and pulegone, its major compound, have shown an antispasmodic effect on rat isolated tracheal and bladder smooth muscles. The suggested mechanism for this effect is calcium channel blockade (23).

3.5. Ocimum basilicum L.

The effect of *O. basilicum* on vascular relaxation was investigated in rats, and it was found that the consumption of the aqueous extract of this plant leads to a reduction in blood pressure and an increase in blood flow (24).

3.6. Origanum majorana L.

The antispasmodic effect of *Origanum majorana* has been investigated and confirmed in various studies. For instance, in a research studied on the ether, ethyl acetate, petroleum, aqueous, methanol, and dichloromethane extracts of *O. majorana*, all the extracts have shown a spasmolytic effect on the isolated jejunum of rabbit (15).

3.7. Thymus vulgaris L.

The antispasmodic effect of *Thymus vulgaris* has been confirmed in several studies. In a

triple-blind clinical trial, the pain-relieving effect of *T. vulgaris* essential oil was compared to that of ibuprofen .in primary dysmenorrhea with 84 subjects. It was found that *T. vulgaris* could relieve the symptoms of primary dysmenorrhea (25). Based on the information obtained from a systematic review, the use of *T. vulgaris* in primary dysmenorrhea could reduce pain and spasm (26). In a study conducted on the tracheal chains of guinea pigs, the macerated and aqueous extracts of *T. vulgaris* were able to inhibit the contraction of tracheal chains. The suggested mechanisms for the spasmolytic effect of the aqueous extract were stimulation of β_2-adrenoceptors, inhibition of histamine H_1 receptors, anticholinergic activity, and blocking of calcium channels (just for macerated extract) (27). In another study about the spasmolytic activity of a solid formulation made by *T. vulgaris* essential oil, it was found that the formulation could inhibit calcium channels in the gastrointestinal smooth muscle of guinea pig (28).

3.8. Teucrium chamaedrys L.

There was no or not enough studies to confirm the antispasmodic effect of *T. chamaedrys.*

4. Discussion

The foundation of traditional medicine has been formed over the centuries from the experience of the ancestors and their information about herbals which can be used to solve the many mysteries behind human diseases (38). In TIM, many herbal medicine and foods have been suggested in order to reduce black bile. The present study was conducted to review plants believed as black bile-reducing agent in a TIM manuscript to provide a

Table 1. Major phytoconstituents (%) of the essential oils reported in black bile-reducing plants of Lamiaceae family.

Plant	Compounds	Percentage (%)	References
Dracocephalum moldavica L.	Citral	31.14 %	(29)
	Geranyl acetate	36.62 %	
	Geraniol	24.31 %	
	Limonene	1.35-19.8 %	
	α-Pinene	14.4 %	
	Carvacrol	7.8 %	
Lavandula stoechas L.	Fenchone	50.29-55.79%	(30, 31)
	Camphor	14.02-18.18%	
	α-Pinene	23.18%	
	D-fenchone	29.28%	
	1,8-Cineole	8.03%	
Melissa officinalis L.	β-Caryophyllene oxide	0.4 -54.1%	(32, 33)
	Geranial	0.5-44.2%	
	β-Caryophyllene	1.2-18.6%	
	Citronellal	1.3-60%	
	Thymol	11.96%	
	Neral	0.8-45.7%	
Mentha pulegium L.	Pulegone	54.4 %	(34)
	p-Menthone	14.0 %	
	Piperitenone	12.8 %	
	Piperitone	3.7%	
	Isopulegone	2.5 %	

Origanum majorana L.	Terpinene-4-ol	32.69%	(35)
	Ɣ-Terpinene	12.88 %	
	α-Terpinene	7.98 %	
	trans-Sabinene hydrate	8.47 %	
	Sabinene	6.21 %	
	α-Terpineol	5.25%	
	trans-Caryophyllene	2.31%	
	(E)-p-Menth-2-en-1-ol	2.25%	
Teucrium chamaedrys L.	β-Caryophyllene	3.9-47.6%	(36)
	Germacrene D	9.5-32.1%	
	α-Humulene	6.7-7.5%	
	δ-Cadinene	3.1- 13.1%	
	Caryophyllene oxide	3.1-12.3%	
	(E)-α-Caryophyllene	33.9%	
	(Z)-β-Farnesene	7.6-12.2%	
	α-Muurolene	15.3%	
	(E)-β-Farnesene	4.3-6.5%	
Thymus vulgaris L.	Thymol	26.18%	(37)
	α-Pinene	8.47%	
	1,3,8-p-Menthatriene	25.16%	
	Limonene	12.37%	
	Linalool	5.64%	

scientific basis for further studies.

Based on the *Qanun of Medicine*, foodstuffs are converted into "Humour" during liver digestion. Four humors are yellow bile, blood, black bile, and phlegm, which are correlated with the four elements: fire, air, earth, and water, respectively. Each of them has a pair of unique qualities; hot and dry, hot and wet, cold and dry, and cold and wet (1). In the human body, some organs are the storage place of some of humours and their most important role is to prevent the entry of a large volume of humours into the blood circulation; otherwise, diseases will occur (1, 2). The quality of black bile is cold and dry. Diseases caused by black bile or the predominance of coldness and dryness together include a wide range of physical and mental disorders such as muscle spasms, which was the focus point of current study. Due to the cold and dry nature of black bile, its excessive accumulation in the body organs causes stiffness, dryness, and constipation. For instance, atherosclerosis is caused by the accumulation of abnormal black bile in the wall of the artery (1). Therefore, with the increase of black bile in muscles, muscle spasm occurs as a result of black bile deposition in the muscle. According to recent studies, the exact etiology of spasm has not been identified yet. A hypothesis proposed muscle dehydration (5). Since the dominance of black bile in any part of the body causes the dryness of that organ, muscle dehydration may be related to the dryness characteristics of black bile.

The studied plants, i.e. *Melissa officinalis* L., *Lavandula stoechas* L., *Ocimum basilicum* L, *Thymus vulgaris* L., *Dracocephalum moldavica* L., *Mentha pulegium* L., *Teucrium chamaedrys* L. and *Origanum majorana* L. are all from the Lamiaceae family. In the TIM literature, they were mentioned as a cure for diseases caused by black bile via reducing black bile in the body and eliminating it. The results showed that these plants have antimicrobial, antioxidant, anti-inflammatory, an-

tidepressant, anxiolytic, cytotoxic, antiplatelet, antispasmodic, hypoglycemic, and hypolipidemic effect (39-42). With a precise checking of results, it was found that all these plants have antispasmodic and spasmolytic effects. These findings suggest that the antispasmodic effect of *Lavandula stoechas* and *Mentha pulegium* may be due to the blocking of Ca^{+2} channels (15, 16). *Melissa officinalis* spasmolytic effect is related to the involvement of muscarinic receptors and calcium channels (21), and for *Thymus vulgaris*, it is due to the stimulation of β-adrenergic receptors and / or blocking of histamine H1 receptors (27). According to TIM, the part of *Ocimum basilicum* L. that is used to reduce black bile is its seed. Despite an extensive search of databases, no information was found on the content of the essential oil of the seeds. However; the antispasmodic effect of the aqueous extract of its leaves has been investigated in studies. Therefore, solving this contradiction requires further studies. Lamiaceae or mint family are known for the presence of essential oils and diterpenoids in many members of the family. A high number of biologically active components from essential oils have been isolated. Based on the historical evidence, these plants have been used by humans since ancient times (43). The plants studied here were from the Lamiaceae family and contain large amounts of essential oil.

Essential oils are complex mixtures of volatile, natural, and low molecular weight compounds, which are obtained by steam or hydro-distillation of plant organs, especially from aromatic plants. They have a strong odor, and protect the plant against bacteria, viruses, fungi and insects. They are synthesized in all parts of the plant, such as the stem, leaves, roots, buds, flowers, twigs, seeds, fruits, wood or bark (44, 45). Since ancient times, herbs and spices have been used as flavoring and preservatives in addition to therapeutic purposes (46). Essential oils have a high absorption rate and can easily enter the body through the skin, oral and pulmonary tissues (47). The dominant constituents of essential oils include monoterpene and sesquiterpene hydrocarbons, and miscellaneous volatile organic compounds (44). Based on studies, essential oils have shown antibacterial, antifungal, antirheumatic, antitussive, antiviral, expectorant, analgesic, sedative, spasmolytic, anti-inflammatory, antioxidant, anticarcinogenic, and blood-circulation-enhancing effects (44-46). The components of the essential oils obtained from the plants are responsible for their biological activities; therefore, the antispasmodic effect of plants is probably due to the presence of the main constituents of essential oils.

Based on the observed results in Table 1, the most represented group of compounds in the essential oils of the studied species are monoterpenoids and sesquiterpenes. Silva et al. have demonstrated monoterpenes carvacrol, citronellal and p-cymene had a relaxing effect on guinea-pig trachea. The possible mechanism for carvacrol that has greater efficacy and potency, among others, is to have an anti-muscarinic effect and/ or agonist effect on beta-adrenergic receptors (48). The study conducted by Norouzi *et al*. showed that pulegone (a monoterpene ketone) significantly decreased spontaneous and induced contractions on the bovine ileum. It has been reported that the relaxation mechanism of pulegone in guinea pig ileum is due to the blocking of Ca^{2+} channels, activation of K^{+} channels and non-competitive antagonism of muscarinic receptors (49). Sadraei *et al*. have shown that both *Melissa officinalis* essential oil and its major compound citral had a spasmolytic effect, inhibiting ileum contraction via depolarization and antagonism effect on muscarinic or serotonergic receptors (50). According to one study conducted by Rehman et al, fenchone relaxed the isolated guinea-pig trachea by activation of K^{+} channels followed by the dual inhibition of PDE and Ca^{+2} channels. Furthermore, by performing molecular docking studies the insight of fenchone binding with Ca^{+2} channel was raised (51). Caryophyllene, a naturally occurring sesquiterpene, was able to relax the tracheal smooth muscle in the isolated organs. The antispasmodic effect of caryophyllene on the isolated tracheal smooth muscle of rats is exerted through voltage-dependent L-type Ca^{+2} channel inhibition (52). Based on the studies, linalyl acetate, which is the ester form of linalool from the group of monoterpenes, has shown antispasmodic effects on the isolated intestines of guinea pigs and rabbits (53, 54).

5. Conclusion and future perspectives

Black bile does not have a fixed definition in the whole body and it appears in a specific form with deposition in each organ. For example, the symptom of increasing black bile in the muscles is muscle spasms. So, they can be considered as a criterion for the presence of black bile in the muscles. In the modern medicine, these plants have an antispasmodic effect; by considering the spasm as a measure of black bile, these plants and reduction of black bile might have some correlation.

The antispasmodic effect of major compounds of the essential oils of the target plants, such as carvacrol, citral, fenchone, pulegune, caryophyllene and linalyl acetate, as well as the plants themselves, has been mentioned. Therefore, by considering these plants as black bile-reducing agents, the hypothesis that the main components of the essential oils play a role in reducing the black bile can be proposed. However, further studies are required to evaluate this theory.

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Conflict of Interest

None declared.

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