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Review Article

Relaxant effect of *Zataria multiflora Boiss L.* and its ingredients on smooth muscles, possible mechanisms and clinical application

Mohammad Reza Khazdair[1], Zahra Gholamnezhad[2,3], Saeideh Saadat[4], Mohammad Hossein Boskabady[2,3*]

1. Cardiovascular Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran
2. Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Physiology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

ABSTRACT

Zataria multiflora Boiss L. (*Z. multiflora*) is belonged to the Lamiaceae family, formerly used for culinary and medicinal purposes. Various pharmacological effects of *Z. multiflora* such as bronchodilation, effect on lung inflammation, cold and gynecology disorders have been reported. A literature search was performed in the following databases: Science Direct, PubMed, Scopus and Google Scholar. The keywords including "*Zataria multiflora* Boiss.", "carvacrol", "thymol", "linalool", "smooth muscle" and "relaxant effects" were searched. The relaxant effects of *Z. multiflora* and its ingredients on different smooth muscles including trachea, vascular, gastrointestinal and urogenital smooth muscle were demonstrated. The relaxant effect of *Z. multiflora* on smooth muscles could be of therapeutic importance, such as bronchodilation in obstructive respiratory disorders, vasodilation in hypertension and reliving digestive or urogenital disorders. The possible mechanisms of the relaxant effect of *Z. multiflora* and its components, mainly carvacrol on smooth muscle such as inhibitory effect on histamine (H_1) and muscarinic receptors, calcium channel blocking effects and stimulatory effect on the beta adrenergic receptor were shown.

Keywords:
Smooth muscle
Relaxant effect
Zataria multiflora Boiss L
Carvacrol
Thymol
Linalool

Introduction

Zataria multiflora Boiss L. (*Z. multiflora*) is belonged to the Lamiaceae family with a woody and fibrous root with 40-80cm height. The leaves of the plant are small, narrow, elliptical and greenish-grey in colors. The pale purple flowers terminate the branches. The seeds are very small and roundish which geographically grows wild only in central and southern Iran, with folk name of Avishan Shirazi (in Persian) (Hosseinzadeh et al., 2000). *Z. multiflora* is used as antiseptic, analgesic, carminative, anthelmintic and antidiarrheal properties in traditional medicine (Pharmacopoeia 2002). It has been reported that the extract of this plant has been able to treat coughs due to colds, bronchitis, laryngitis, disorders of the oral cavity and as an antibacterial agent in Iranian traditional medicine (Avicenna 1985; Mozaffarian, 1996).

Z. multiflora has similar chemical and pharmacological properties to *Thymus vulgaris* (another species of Lamiaceae family), the well-known investigated medicinal plant (Amin 1991). Identified constituents of

* Corresponding author: Mohammad Hossein Boskabady, boskabadymh@mums.ac.ir
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T. vulgaris are phenols, flavonoids, saponins, tannins and particularly terpenes such as thymol and carvacrol (ESCOP, 1996). *Z. multiflora* essential oil (ZMEO) due to antioxidant, antifungal and antimicrobial properties was used for producing functional foods as an effective agent on preservation of the foods (Kordsardouei et al., 2013; Mortazavi Moghaddam et al., 2020). The effects of ZMEO on some hematological parameters including red blood cell (RBC) count and hematocrit had been investigated. The plant significantly enhanced the respiratory burst activity of neutrophils while it had a moderate effects on RBC and hematocrit (Sheikhzadeh et al., 2011). The hydroalcoholic extract of *Z. multiflora* on the animals with duodenal ulcer significantly reduced ulcerated area and index (Minaiyan et al., 2005).

The antioxidant effects of *Z. multiflora* (Fatemi et al., 2012) and the beneficial effect of ZMEO on cognitive function and mental abilities in an animal models of Alzheimer (Majlessi N 2012) and Parkinson diseases (Khazdair et al., 2020b) have been demonstrated. Therapeutic effect of *Z. multiflora* on chronic cough in children was reported (Mortazavi Moghaddam et al., 2020). The effects of *Z. multiflora* on bronchial inflammation and cough due to colds in the traditional medicine have been reported (Mozaffarian, 1996). The antitussive effect of the plant was also been suggested (Afzali et al., 2003). The therapeutic effect of *Z. multiflora* in sulfur mustard induced lung injuries has been demonstrated too (Khazdair et al., 2020a; Khazdair et al., 2020c; Mostafavi and Shasavari 2005). Treatment of animal model of chronic obstructive pulmonary disease (COPD) with the plant extract leads to reduced total white blood cell (WBC), the neutrophils counts, malondialdehyde level and eosinophils count but increased the level of interleukin (IL)-8 in the serum (Boskabady and Gholami Mhtaj 2014). In addition, *Z. multiflora* has a protective effects on serum levels of nitric oxide, phospholipase A2 and total protein of ovalbumin-sensitized guinea pigs (Boskabady et al., 2014). The effect of the plant extract on Th1/Th2 balance on both sensitized animals and human mononuclear cells were also showed previously (Boskabady et al., 2013). The effect of the plant and its constituent, carvacrol on asthma and COPD in clinical studies were also reported (Alavinezhad et al., 2020; Alavinezhad et al., 2017a; Ghorani et al., 2020).

In previous studies the effect of carvacrol, on tracheal responsiveness, inflammatory cytokines and WBC count (Boskabady and Jalali 2013) as well as its protective effect on endothelin level of sensitized animal (Jalali et al., 2013) had been shown which may confirm the immune regulatory and anti-inflammatory properties of the plant. The effect of carvacrol on reduced paw edema and some inflammatory cytokine such as, prostaglandin E2 and IL-1β was demonstrated, but it was not changes significantly tumor necrosis factor-α. Carvacrol also reduced the cyclooxygenase-2 and IL-1β mRNA expression but did not reduce the complete Freund's adjuvant induced paw edema and IL-10 (da Silva Lima et al., 2013). In addition the results showed that carvacrol enhanced the IL-10 mRNA expression and level of IL-10 as an anti-inflammatory cytokine in the inflamed paw in animals (da Silva Lima et al., 2013). The aim of the current study is to review the relaxant effect of *Z. multiflora* and its ingredients on smooth muscles as well as possible mechanisms and clinical application of this effect.

Material and methods

Different databases including, Science Direct, PubMed, Scopus and Google Scholar were searched from beginning of 1985 to September 2020. The available articles (2144) were obtained by searching the following MESH terms in title and abstract including (*Z. multiflora*) AND (carvacrol) AND (thymol) AND (linalool) AND (smooth muscle) AND (relaxant effect) AND (animal or human). Relevant information was collected from articles that reported the effects of *Z. multiflora* and its constituents on the smooth muscle relaxant effects, in English were included. Unrelated studies, letter to the editors, review articles and non-English articles were not considered.

Plant constituents

Z. multiflora essential oil analyzed by GC/FID and GC/MS showed 56 compounds which the major components are thymol, carvacrol, linalool and p-cymene (Hadian et al., 2011). The main components of *Z. multiflora* essential oil were thymol (42.46%), carvacrol (16.85%) and p-cymene (10.62%), (Figure 1) (Aida et al., 2015). The result of another study showed that the major compounds of *Z. multiflora* were thymol (61.8%), carvacrol (10.5%), p-cymene (7.5%) and gamma-terpinene (4.4%) (Fatemi et al., 2012). The chemical structure of the major components of *Z. multiflora* covered in the present review paper is listed in Figure 2.

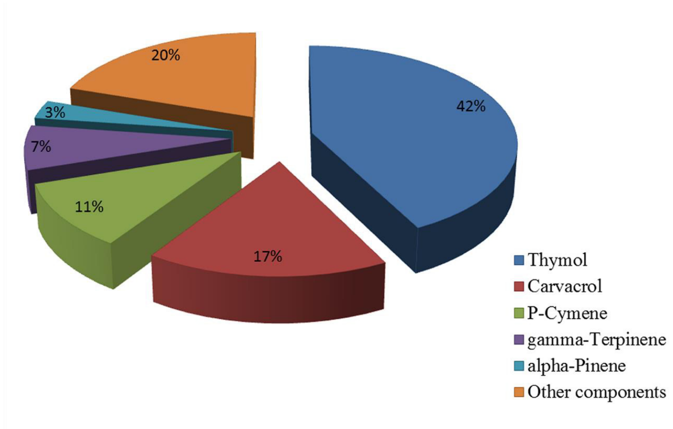

FIGURE 1. The main components of *Z. multiflora* Boiss essential oil (Aida et al., 2015).

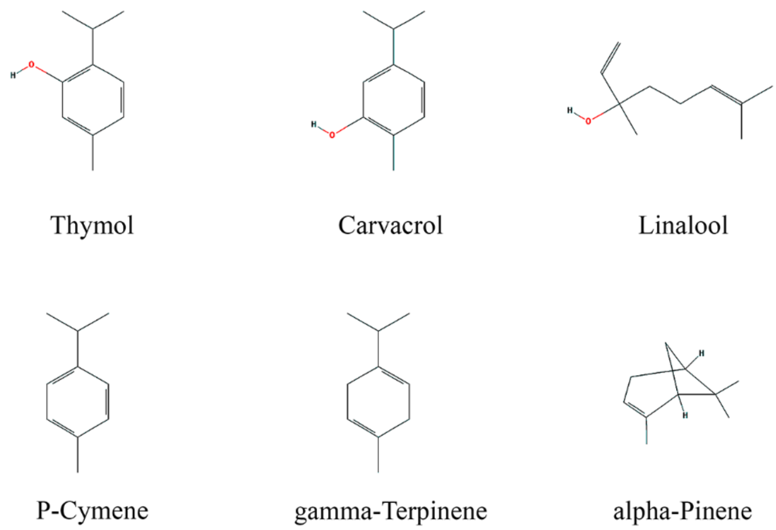

FIGURE 2. The chemical structure of the major components of *Z. multiflora* including thymol ($C_{10}H_{14}O$), carvacrol ($C_{10}H_{14}O$), linalool ($C_{10}H_{18}O$; $(CH_3)_2C=CH(CH_2)_2C(CH_3)(OH)CH=CH_2$), p-cymene ($C_{10}H_{14}$; $CH_3C_6H_4CH(CH_3)_2$), gamma-terpinene ($C_{10}H_{16}$) and alpha-pinene ($C_{10}H_{16}$).

Relaxant effects of plant extracts

Tracheal smooth muscles (TSM)

The therapeutic effect of *Z. multiflora* and its main ingredient, carvacrol in respiratory disorders such as asthma (Alavinezhad et al., 2017b; Alavinezhad et al., 2018) chemical war victims (Khazdair et al., 2018a; Khazdair and Boskabady, 2019a; Khazdair and Boskabady, 2019b; Khazdair et al., 2018b; Mostafavi and Shasavari, 2005) and its antitussive effect were suggested (Afzali et al., 2003), which could be due to the relaxant effect of the plant extracts on airway smooth muscle leading to bronchodilation. The relaxant effect of *T. vulgaris* (another species of the Lamiaceaefamily) on TSM have also been shown (Boskabady et al., 2006). In addition the relaxant effects of *Thymus* extracts and theophylline on precontracted TSM of guinea pig with 60mmol/l KCl and 10μmol/l methacholine showed significant relaxant effects similar to theophylline (Keyhanmanesh et al., 2014). Administration of hydro-ethanolic extract of *Z. multiflora* reduced tracheal responsiveness in animal model of asthma (Boskabady et al., 2014) and COPD (Gholami Mahtaj et al., 2015) which could be due to the TSM relaxant effect.

Gastro-intestinal and urogenital smooth muscle

ZMEO (0.125, 0.25, 0.5, 1 and 2mg/ml) reduced the tonic and phasic contraction induced by 80mM KCl and 320nM acethylcholine (Ach) of ileum smooth muscle. This findings indicate its probable usefulness for control of uterus spasm (Sadraei et al., 2003). Spasmolytic ef-

TABLE 1: The relaxant effect of the extract of *Z. multiflora* on different types of smooth muscle

Smooth muscle type	Extract	Effects	Reference
Trachea	Aqueous extracts of *Z. multiflora*	Protective effects on increased tracheal responsiveness to methacholine and OVA	(Boskabady et al., 2014)
	Different fraction *T. vulgaris*	Relaxant effect on TSM	(Boskabady et al., 2006; Keyhanmanesh and Boskabady, 2012)
	Aqueous extracts of *T. vulgaris*	Relaxant effect on TSM of guinea-pig	(Alavinezhad et al., 2017b)
Gastrointestinal tract	*Z. multiflora* hydroalcoholic extract	Spasmolytic effect on ileum	(Naseri, 2003)
Urogenital tracts	*Z. multiflora* essential oil	Control of uterus spasm	(Sadraei et al., 2003)
	Hydroalcoholic extract	Relaxant effect on rat uterus	(Gharib Naseri et al., 2010)
	Z. multiflora essence	Improved premenstrual syndrome severity score	(Sodouri et al., 2013)
	Z. multiflora essence	Decreased dysmenorrhea severity	(Iravani, 2009)
Cardiovascular	Aqueous extract of *T. vulgaris*	Vasorelaxant and antihypertensive effects	(Kensara et al., 2013)

TSM: tracheal smooth muscle

fect of *Z. multiflora* hydro alcoholic leaf extract (ZHLE) induced by KCl, barium chloride ($BaCl_2$) and oxytocin contractions in rat ileum and uterus were also observed (Gharib Naseri et al., 2010; Naseri, 2003). *Z. multiflora* hydro alcoholic extract also significantly reduced contracted of the ileum by 60mM KC, 0.05μg/ml Ach and 4mM $BaCl_2$ in a dose dependently manner. The relaxant effects of the extract on KCl and $BaCl_2$ induced contraction were similar but the its effect on Ach-induce contraction was greater than other stimulants (Gharib Naseri et al., 2010).

In a double-blinded, randomized trial, 88 eligible students for premenstrual syndrome (PMS) were randomly divided to intervention and placebo group. One group received pearls containing 20mg of *Z. multiflora* essence (4 pearls each day) for two menstrual cycles, 7days before menstruation and another group received placebo. The results showed no significant difference between intervention groups regarding the severity of PMS. However a significant improvement in PMS severity score between intervention and placebo groups was observed after the intervention. The pearls containing *Z. multiflora* essence could not decrease significantly the PMS severity and the frequency of symptoms (Sodouri et al., 2013).

In traditional medicine *Z. multiflora* was recommended for the relief of dysmenorrhea. Therefore, in a clinical trail 108 adolescents (18-24 years) who complained of primary dysmenorrhea were randomly assigned into three groups: the placebo group (n=36), ZMEO 1% (n=36) and ZMEO 2% (n=36). Mean dysmenorrhea severity was significantly decreased in two treated groups compared to placebo group after intervention. In the 1% ZMEO, 2% ZMEO and placebo groups, 41.8%, 39.9% and 66.7% of patients needed to use other medications to relief symptoms (Iravani, 2009).

Vascular smooth muscle

The aqueous extract of *T. vulgaris* showed antihypertensive effects on rats which correlated with histopathological changes. This effect might be due to the direct smooth muscle relaxant and vasorelaxant properties of the plant (Kensara et al., 2013). The relaxant effect of the extract of *Z. multiflora* on different types of smooth muscle were summarized in the Table 1.

Relaxant effects of the plant constituents

Carvacrol

TSM

The relaxant effect of cumulative concentrations of carvacrol (0.02, 0.04 and 0.08ml) on TSM was shown which was even more potent than theophylline at used concentrations (Boskabady and Jandaghi 2003). Relaxant effects of three monoterpens including, carvacrol, citronellal and p-cymen on contracted tracheal smooth-muscle rings by carbachol (10^{-6} M) in the present and absent of endothelium were investigated. Efficacy of carvacrol was greater than other monoterpens, citronellal and p-cymen. E_{max} of carvacrol (10^{-4} M) was 100% in the presence and absence of endothelium, while E_{max} of citronellal were 79.6±10.2 and 90.2±5.0 in the presence and absence of endothelium and E_{max} of p-cymen were 69.0±9.4 and 79.0±4.4 in the presence and absence of endothelium. This results indicat the relaxant effects of these monoterpens, which efficacy of carvacrol was greater than of the other monoterpens (Silva et al., 2014). The effect of carvacrol on reduction of tracheal responsiveness in animal model of asthma (Boskabady et al., 2014) and COPD (Gholami Mahtaj et al., 2015) was also documented which may be due to the relaxant effect of carvacrol on TSM.

The relaxant effect of the other plant with carvacrol content has been also shown which may confirm the relaxant effect of carvacrol on TSM. The relaxant effects of the aqueous-ethanolic extract of *Achillea wilhelmsii* (4, 6 and 8mg/ml) on tracheal chains of guinea pigs were examined (Boskabady et al., 2009). The main components of the oil of *A. wilhelmsii* were carvacrol (25.1%). The bronchodilatory effects of *Carum copticum* seeds boiled extract (0.125 and 0.25 ml/kg of 10 g%) on the asthmatic patients airways (Boskabady et al., 2007) and the relaxant effects of its fractions on tracheal chains of guinea pigs were showed (Boskabady et al., 2003). The essential oil of the seeds of this plant contains terpinene, p-cymene, α-pinene, β-pinene, thymol and carvacrol. The relaxant effects of aqueous extract of *Ferula asafoetida* (2, 5 and 10mg/ml) on TSM of animals were reported (Bayrami et al., 2013). Thymol (10%) and carvacrol (less than 1%) are the constituents of this plant. The bronchodilatory effect of orally administered boiled extract of *Portulaca oleracea* (0.25 ml/kg of 5% boiled extract) in asthmatic patients (Malek et al., 2004) and the relaxant effects of boiled and aqueous extract of this plant on TSM of the animals were demonstrated (Boskabady et al., 2004a).

The bronchodilatory effects of boiled extract of *Nigella sativa (*50 and 100mg/kg) which is also contain carvacrol in asthmatic patients was examined which significantly enhanced all measured pulmonary function tests (Boskabady et al., 2010a). The relaxant effects of different extract and fractions of this plant on TSM of animals were also shown (Boskabady and Sheiravi 2002; Boskabady et al., 2008; Boskabady et al., 2004b).

Vascular smooth muscle

Carvacrol, is an agonist for ankyrin transient receptor potential (TRP) channel (TRPA1) and vanilloid TRP cation channel (TRPV3), (Xu et al., 2006). Functional TRPV3 channels are present in the endothelium of cerebral and cerebellar arteries (Earley et al., 2009). TRPV3 channels are stimulated by agents including vanillin, thymol and eugenol (Xu et al., 2006). Carvacrol also causes Ca^{+2} influxes in the endothelium via TRPV3 channels. Increases in endothelial intracellular Ca^{+2} activate intermediate conductance Ca^{+2} activated K channel (IK_{Ca}) and low-conductance Ca^{+2} activated K channel (SK_{Ca}), hyperpolarizing the plasma membrane of endothelial cells and principal smooth muscle. Carvacrol induced vasodilation with hyper-polarization of the smooth muscle cell plasma membrane (Earley et al., 2009). Carvacrol at 1, 10 and 20µg/kg did not affect heart rate, mean arterial pressure, systolic and diastolic blood pressures but its 100µg/kg, decreased these parameters in the anesthetized rats. Inhibitory effects of carvacrol on N((omega))-nitro- L-arginine methyl ester (L-NAME)-induced hypertension was observed which may be due to inhibitory effect on cardiac L-type calcium channel of carvacrol (Aydin et al., 2007).

Gastro-intestinal smooth muscle

It has been reported that carvacrol (10^{-2}, 10^{-3} and 10^{-4} M) decreased Ach (10^{-4}M)-induced contraction in isolated rat's ileum. The antispasmodic effect of carvacrol (10^{-5}, 10^{-4} and 10^{-3}M) on contracted smooth muscle by Ach (10^{-3}M) was also reported (Faghihi et al., 2017).

Thymol

TSM

Four fractions of *T. vulgaris* (0.4, 0.8, 1.2 and 1.6 g%), its main constituent, thymol and theophylline (0.2, 0.4, 0.6 and 0.8mM) demonstrated relaxant effect on TSM. The relaxant effect of alcoholic fraction was less than that of theophylline but n-hexane fraction showed a relaxant effect higher than that of theophylline (Keyhanmanesh and Boskabady 2012). However, previous studies did not show any relaxant effect for thymol on TSM

TABLE 2: The relaxant effect of ingredients of *Z. multiflora* on different types of smooth muscle

Constituent	Plants	Tissue	Effects	Reference
Carvacrol		Trachea	Relaxant (bronchodilatory) effect	(Boskabady and Jandaghi, 2003)
		Gastro-intestinal tracts	Antispasmodic effects on isolated rat's ileum	(Faghihi et al., 2017)
Plant containing carvacrol	*Achilleawilhelmsii*	Trachea	Relaxant effect	(M.H. Boskabady, 2009)
	Carumcopticum		Broncho-dilatory effects	(Boskabady et al., 2007)
	Ferula Asafoetida		Relaxant effects	(Bayrami et al., 2013)
	Portulacaoleracea		Bronchodilatory effect in asthmatic patients	(Malek et al., 2004)
	Portula Caoleracea		Broncho-dilatory effect on tracheal chains of guinea pigs	(Boskabady et al., 2004a)
	Nigella sativa		Bronchodilatory effect in asthmatic patients	(Boskabady et al., 2010a)
	Nigella sativa		Relaxant effect on TSM of guinea pigs	(Boskabady and Sheiravi, 2002; Boskabady et al., 2008; Boskabady et al., 2004b)
Thymol			Did not show any relaxant effect	(Boskabady et al., 1998)
		Gastro-intestinal tracts	Inhibited excitatory effect on circular smooth-muscle strips	(Hejazian et al., 2013)
			Relaxed the spontaneous contractile activity of the smooth-muscle strips from guinea pig stomach	(Beer et al., 2007)
		Cardiovascular	Inhibited the spontaneous contractile activity on circular smooth-muscle strips from vena portae	(Beer et al., 2007)
Linalool			Induced relaxations in the mesenteric artery	(Anjos et al., 2013)

TSM: tracheal smooth muscle

(Boskabady et al., 2011; Boskabady et al., 1998).

Gastro-intestinal and urogenital smooth muscle

Effects of thymol (3×10^{-8} and 2×10^{-7} M) on the contractile activity of circular smooth-muscle strips (SMA) from guinea pig stomach induced a strong and clear excitatory effect but the higher concentration of thymol (10^{-6}–10^{-4} M) inhibited of this excitatory effect. Therefore, the relaxant effect of thymol is occurred at concentrations of 3×10^{-7}–2×10^{-6} M but its spasmolytic effect was also observed at concentration of 10^{-6} M. In addition thymol at concentration of 10^{-4} M fully (100%) inhibits the spontaneous contractile activity of SMAs from guinea pig stomach (Beer et al., 2007). The result of other study showed that Ach (10^{-4}M) in the presence of low concentration (10^{-4} and 10^{-5}M) of thymol induced contraction in isolated rat's ileum while concentration of 10^{-3}M of thymol significantly inhibited smooth muscle contraction (Hejazian et al., 2013). Thymol also showed an agonistic effects on the α_1-, α_2- and β-adrenergic receptors of smooth-muscle from guinea pig stomach (Beer et al., 2007).

Vascular smooth muscle (VSM)

The effects of thymol (3×10^{-8} and 2×10^{-7} M) on the spontaneous contractile activity (SCA) on SMA from vena portae induced a strong excitatory effect on SCA of SMS, which reached 15% of the maximum contractile activation of SMS by Ach (10^{-5}M). However, the response curve effect of thymol in higher doses between (10^{-6}–10^{-4} M) showed total inhibition of SCA of SMS. The inhibition of SC of SMS occurs more slowly than the excitatory effects (Beer et al., 2007).

Linalool

Oral administration (p.o.) of linalool the other monoterpene constituents of *Z. multiflora* at dose 200mg/kg reduced blood pressure. This effect is probably due to a direct effect on the Vascular smooth muscle (VSM) leading to vasodilation. Linalool ($6.4\cdot10^{-6}$ to $6.4\cdot10^{-3}$M) induced relaxation in the mesenteric artery precontracted with 10µM phenylephrinine (Anjos et al., 2013). The relaxant effects of constituents of *Z. multiflora* on different types of smooth muscle were shown in the Table 2.

Possible mechanisms of the relaxant effect of Z. multiflora and its ingredients

The relaxant effect of the extract of *Z. multiflora* and its constituents could be due to several mechanisms including inhibitory effect on muscarinic and/or on histamine (H_1) receptors, stimulatory effect on β2-adrenoceptor, calcium channel blocking effect and other mechanisms which will review in this section.

Inhibitory effect on muscarinic receptors

The rightward shifts in concentration–response curves, obtained in the presence of the extract of *Z. multiflora*, higher EC_{50} and lower maximum response to methacholine (MRM) compared to that of saline in non-incubated tracheal smooth muscle indicated a functional antagonistic effect of the extract at muscarinic receptors of guinea pig TSM (Boskabady et al., 2012a). The rightward shift in methacholine–response curves obtained in the presence of *Z. multiflora* extract (0.5, 1 and 2ml) compared to that of saline, significantly improved MRM and increasd EC_{50} obtained in the incubated tracheal chains with propranolol and chlorpheniramine relative to those of non-incubated TSM showed possible competitive antagonistic effects of the hydro-ethanolic extract on muscarinic receptors (Boskabady et al., 2012a). These findings indicate that the relaxant effect of the plant is due to inhibition of muscarinic receptor in smooth muscle (Loenders et al., 1992).

The rightward shift in methacholine-response curve was also obtained in the presence of carvacrol (0.1, 0.2 and 0.4mg/ml) compared to the curve obtained in the presence of saline. The significant improvement in MRM and increase EC_{50} obtained in incubated TSM with 1mM chlorpheniramine and 1mM propranolol showed possible competitive antagonistic effects of carvacrol on muscarinic receptors of tracheal smooth muscle (Boskabady et al., 2011).

Inhibitory effects on histamine (H1) receptors

The concentration–response curves of TSM to histamine obtained in the presence of the extract of *Z. multiflora* (2.5, 5 and 10µg/ml) and chlorpheniramine (10nM) showed significant rightward shift compared to histamine curves produced in the presence of saline, in incubated tissues with 1.4µM indomethacin and 1µM propranolol. The EC_{50} values of histamine obtained in the presence of chlorpheniramine and the extracts were significantly higher than that of saline (Jafari et al., 2011). The rightward shifts in histamine response curves in the presence of the aqueous-ethanolic extract, greater EC_{50} and obtaining of maximum contraction effect to histamine compared to that of saline in the incubated tissues with indomethacin indicated a competitive antagonistic effect of *Z. multiflora* at histamine H_1 receptors of guinea-pig TSM (Jafari et al., 2011). In addition in a similar study, concentration–response curves to histamine in the presence of the *Z. multiflora* extract (2.5, 5 and 10µg/ml), carvacrol (1, 2 and 4µg/m) and chlorpheniramine (10nM) in TSM showed a remarkable rightward shifts in histamine curves compared to the curve produced in the presence of saline. The EC_{50} of histamine in the presence of chlorpheniramine extract and carvacrol was significantly higher than that of saline. In addition the results of this study showed concentration dependent inhibitory effect on histamine (H_1) receptors for the extract and carvacrol (Boskabady et al., 2012b).

Stimulatory effect on β2-adrenoceptor

The adrenergic stimulatory effects of *Z. multiflora* hydro-ethanolic extract were confirmed in incubated TSM with propranolol (Jafari et al., 2011). The left ward shifts in beta-adrenergic agonist (isoprenaline) response curves obtained in the presence of aqueous-ethanolic extract (0.5, 1 and 2µg/ml), lower EC_{50} and obtaining of maximum relaxation effect to isoprenaline compared to that of saline showed possible competitive stimulatory effect of the planthydro-ethanolic extract on β-adrenoceptors of guinea pig TSM. The (CR-1) values obtained in the presence of the extract were negative but that of β-adrenergic antagonist (propranolol) was positive which supported the stimulatory effect of the extract on β-adrenoceptors of guinea pig TSM (M.H. Boskabady 2009).

In a similar study also cumulative log concentration-response curves to isoprenaline obtained in the presence of the extract (0.5, 1 and 2µg/m) and carvacrol (0.1, 0.2 and 0.4µg/ml) in TSM showed leftward shift. While, the curve of propranolol showed rightward shift compared to isoprenaline curves in the presence of sa-

TABLE 3: Possible mechanisms of the relaxant effect of *Z. multiflora* and its ingredients

Mechanism	Tissue	Solution	Reference
Muscarinic receptors inhibitory	Trachea	*Z. multiflora* hydro-ethanolic extract (0.5, 1 and 2 ml)	(Boskabady et al., 2012a)
		Carvacrol (0.1, 0.2 and 0.4 mg/ml)	(Boskabady et al., 2011)
Histamine (H_1) receptor inhibitory		*Z. multiflora* (2.5, 5, and 10 µg/ml)	(Jafari et al., 2011)
		Carvacrol (1, 2, and 4 µg/ml)	(Boskabady et al., 2012b)
β2-adrenoceptors stimulatory		*Z. multiflora* (0.5, 1, and 2 µg/ml)	(M.H. Boskabady 2009)
		Carvacrol (0.1, 0.2, and 0.4 µg/ml)	(Boskabady et al., 2010b)
	Gastrointestinal	Thymol 3×10^{-7}–2×10^{-6} M	(Beer et al., 2007)
		Thymol 10^{-6}–10^{-4} M	(Beer et al., 2007)
Calcium channels blocking		*Z. multiflora* (0.125, 0.25, 0.5, 1 and 2 mg/ml)	(Naseri, 2003)
	Urogenital tracts	*Z. multiflora* essential oil extract (2 mg/ml)	(Gharib Naseri et al., 2010)
	Cardiovascular	Carvacrol	(Aydin et al., 2007)
Oxytocin receptors interacted and/or calcium channels blocking.		*Z. multiflora* essential oil extract (2 mg/ml)	(Gharib Naseri et al., 2010)
Potassium channels (ik_{ca}) and (sk_{ca}), activating		Carvacrol	(Earley et al., 2009)

line in non-incubated and incubated tissues with chlorpheniramine. These results indicated the stimulatory effect of the extract of *Z. multiflora* and carvacrol on β2–adrenoceptors. In addition isoprenaline EC_{50} obtained in the presence of propranolol was remarkably higher than that of saline. However, the EC_{50} obtained in the presence of all concentrations of the extract and carvacrol was significantly lower than that of saline. Lower values of EC_{50} obtained in the presence of the different concentrations of the aqueous-ethanolic extract and carvacrol compared to that of saline confirmed the stimulatory effect on β2-adrenoceptors (Boskabady et al., 2010b). In addition an agonistic effect have been shown for thymol on *β*-adrenergic receptors of smooth-muscle from guinea pig stomach (Beer et al., 2007).

Calcium channel blocking effect

The inhibitory effect of ZHLE on voltage dependent calcium channels in ileum smooth muscle was shown (Gharib Naseri et al., 2010; Naseri, 2003). Relaxant effects of *Z. multiflora* extracts in the presence of 0.5µM atropine, 0.5µM Ach and 30mM KCl in uterus muscle showed in responses to the KCl but not to the Ach. The extract (2mg/ml) significantly reduced uterus contraction which is more potent than the atropine effect (Gharib Naseri et al., 2010). These findings my suggest a calcium channels blocking effect for the extract of *Z. multiflora*, because KCl induced muscle contraction is mediated by voltage dependent calcium channel.

Another study also demonstrated that 300µM thymol increases the depolarization-induced release of calcium from the sarcoplasmic reticulum in fast-twitch muscles of rodents by acting directly on the ryanodine receptors calcium release channels (Szentesi et al., 2004). Carvacrol via TRPV3 channels in the endothelium increased the endothelial intracellular Ca^{+2} which activated K channels (IK_{Ca}) and (SK_{Ca}), and lead to hyperpolarizing the plasma membrane of endothelial cells and principal smooth muscle (Earley et al., 2009). Inhibitory effects of carvacrol on L-NAME induced hypertension due to cardiac L-type calcium channel blocking action was also observed which support the blocking effect of car-

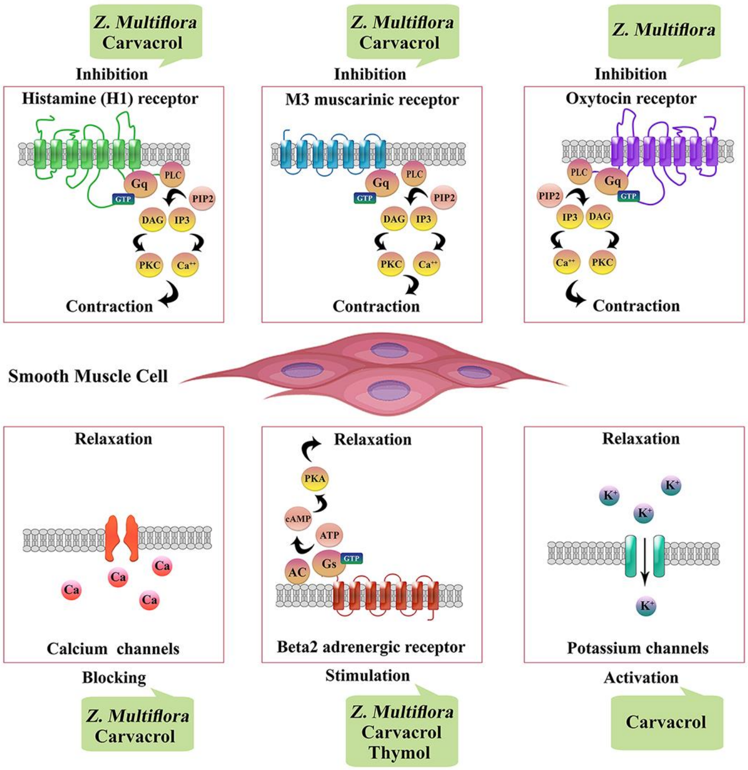

FIGURE 2. Possible molecular mechanisms of the relaxant effect of *Z. multifloraa* and its constituents. AC: Adenylyl cyclase; ATP: Adenosine triphosphate; cAMP: Cyclic adenosine monophosphate; Ca^{++}: calcium; DAG: Diacylglycerol; GTP: guanosine triphosphate; IP3: Inositol trisphosphate; K^{+}: potassium; PIP2: phosphatidylinositol biphosphate; PKA: Protein kinase A; PKC: protein kinase C; and PLC: phospholipase C.

vacrol on calcium channel (Aydin et al., 2007). Possible mechanisms of the relaxant effect of *Z. multiflora* and its ingredients were summarized in the Table 3.

Clinical applications

The relaxant effect of *Z. multiflora* on TSM could indicate the therapeutic potential of the plant in the treatment of airway obstructive disorders including asthma and COPD. In fact, the relaxant effect of *Z. multiflora* in airway obstruction in chemical war victims had been shown (Khazdair et al., 2020a; Khazdair et al., 2020c; Mostafavi and Shasavari 2005), which support this suggestion. The bronchodilatoion effect of *Z. multiflora* comparable to theophylline syrup in asthmatic patients was also reported (Boskabady et al., 2020). These findings also suggest therapeutic potential for the plant and its constituent, carvacrol in obstructive airway diseases.

The relaxant effects of *Z. multiflora* on visceral smooth muscle may cause attenuation of symptoms of PMS and dysmenorrhea severity. In fact in a clinical study, ZMEO decreased dysmenorrhea severity in the intervention patients (Iravani, 2009). Therefore, this plant could be of therapeutic value in urogenital disorders. Moreover the relaxant effects of *Z. multiflora* on PMS in a double-blinded, randomized trial were observed which support the clinical effect of the plant on urogenital disorders (Sodouri et al., 2013). Relaxant effects of *Z. multiflora* essential oils on contracted smooth muscles such as ileum and uterus spasm were demonstrated (Sadraei et al., 2003). Based on these findings the therapeutic effect of the plant on gastrointestinal disorders was also suggested.

The plant constituents showed vasodilatory effect on vascular smooth muscle cell which may indicate its therapeutic effect on hypertension. Vasodilatory effect of carvacrol on hyperpolarized smooth muscle cell plasma membrane (Earley et al., 2009) and its effect on heart rate, systolic, diastolic and mean blood pressures in anesthetized rats (Aydin et al., 2007) were shown. The relaxant effects of thymol on the SCA on SMA from vena portae have been reported (Beer et al., 2007). Oral administration of linalool reduced blood pressure which could be due to a direct effect on the vascular smooth muscle leading to vasodilation (Anjos et al., 2013). Therefore the therapeutic effect of *Z. multiflora* and its constituents on cardiovascular disorders especially on hypertension were suggested. However, further clinical studies are required to elucidate various clinical effects of *Z. multiflora* and its constituents.

Possible molecular mechanisms of the relaxant effect of *Z. multiflora* and its constituents were shown in Figure 3.

Conclusion

The relaxant effects of *Z. multiflora* and some of its constituents on tracheal, gastrointestinal, urogenital and vascular smooth muscle was reviewed which indicated a relatively potent relaxant effect or the extract and constituents of the plant on various types of smooth muscles. The relaxant effect of *Z. multiflora* on smooth muscle suggests its possible clinical applications as bronchodilator, vasorelaxant or similar therapeutic effects on other smooth muscle types. However, further clinical studies are needed to elucidate these clinical applications for the plant extracts and its constituents. The possible mechanisms for the relaxant effect of *Z. multiflora* and its constituents on smooth muscle including inhibitory effect on muscarinic and histamine (H_1) receptors, stimulator effect on β-adrenoceptor and calcium channel blocking effect were also indicated.

Acknowledgments

Not applicable

Conflicts of interest

The author(s) declare no conflicts of interest in this study.

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