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Spasmolytic activity of pulegone on the isolated bovine ileum contractions

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Abstract

Gastrointestinal dysmotility is important in ruminants. Pulegone is a natural monoterpene ketone obtained from the essential oil of different plants. It has been reported that pulegone has an antibacterial, antifungal, and anti-histamine effect. The present study investigated the effects of pulegone on bovine ileum smooth muscle contractions. The experiment was performed on the circular smooth muscle of ileum samples taken from slaughtered bovine in the organ bath. Seven cumulative concentrations of pulegone from 1 to 1000 µg ml^{-1} were added to tissue samples. The solution used was Tyrode's solution aerated with a mixture of 95% oxygen and 5% carbon dioxide, and the temperature was set at 37 °C. The effect of pulegone on baseline contractions and three induced contractions with barium chloride ($BaCl_2$), potassium chloride (KCl), and carbachol (Cch) was investigated. The results revealed that pulegone significantly inhibits spontaneous, as well as all spasmogen-induced contractions. Pulegone was able to relax the contractions caused by Cch, $BaCl_2$, and KCl (20) at 30 µg ml^{-1}. The anti-spasmodic properties of pulegone can be employed for the treatment of intestinal spasms or hypermotility.

Key words: Spasmolytic, Pulegone, Ileum, Bovine

Introduction

Principal functions of the alimentary or gastrointestinal tract including digestion and absorption of food and the excretion of waste (Constable et al. 2017). There are four major modes of gastrointestinal dysfunction that are included abnormality of secretion, motility, digestion, or absorption (Constable et al. 2017). Gastrointestinal dysmotility is an important part of alimentary problems etiology in bovine that is included in two forms: increased and decreased motility (Constable et al. 2017). An increase in irritability in a particular segment of intestine, increases its motor activity and disrupts the natural downward gradient of the movements leading to the passage of the contents from the esophagus to the rectum. Some stimulants may increase the intestinal motor activity and cause cramps, abdominal pain, and colic by causing problems such as diarrhea syndrome (Fecteau et al. 2017, Navarre and Roussel. 1996). Herbal products are economically viable in ruminants which can be used as adjunctive or alternative therapies.

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Different parts of plants for example leaves, seeds, roots, and flowers have been used in ethnoveterinary medicine. Essential oils, tannins, and saponins can decrease or increase the gastrointestinal motility of ruminants (Mendel et al. 2017). Studies showed the useful effects of extracts and essential oils of medical plants on digestive problems of ruminants for example gastrointestinal inflammation, diarrhea, colic, and impaired digestion (EL Mahdy et al. 2019). Given the importance of the topic, various studies have been conducted in this field. In one recent study, the effect of *Bidens tripartita* extract on pig jejunum movements' activity has been shown that this extract has an excellent ability to increase intestine movements (Mendel et al. 2020).

Pulegone is a natural monoterpene ketone obtained from the essential oil of different plants such as *Mentha species* and *Nepeta cataria* (Jalilzadeh and Maham. 2013). It has been reported that Pulegone has an antibacterial, antifungal, and anti-histamine effect (Gong et al. 2021, Amalich et al. 2016), and in Turkey, plants with high pulegone content have been used to treat stomach disorders (Baser et al. 1998). In a new study, the anti-inflammatory effect of Pulegone has been proven to reduce the production of cytokines (Yang et al. 2019). One study showed that *Mentha pulegium* contains large amount of pulegone and has an inhibitory effect on the contractile activity of rat myometrium (Soares et al. 2005). In another study, the action mechanism of the monoterpenes (+)-pulegone and 4-terpinyl acetate showed that the relaxation produced by pulegone in isolated guinea-pig ileum occurs via blockade of Ca^{2+} channels, activation of K^{+} channels, and non-competitive antagonism of muscarinic receptors (Andrade et al. 2013).

The plants seem to have good potential for the prevention and treatment of gastrointestinal problems (Mendel 2016 a, b, Michel et al. 2003). Plants effects on gastrointestinal motility in ruminants were unknown, and Dys-motility is a vital part of the ruminant gastrointestinal disease's pathophysiology. Therefore, natural treatment of these problems are essential in animals. The present study investigated the effect of pulegone on the contractility of bovine ileum smooth muscle.

Materials and Methods

Chemicals. Pulegone, carbachol chloride (Cch), and acetylcholine chloride (Ach) obtained from Sigma (St Louis, USA). Potassium chloride (KCl), sodium dihydrogen phosphate (NaH_2PO_4), calcium chloride ($CaCl_2$), sodium chloride (NaCl), glucose, magnesium chloride ($MgCl_2$), sodium bicarbonate ($NaHCO_3$), barium chloride ($BaCl_2$) and dimethyl sulfoxide (DMSO) were purchased from Merck (Darmstadt, Germany).

Tissue samples collection. Holstein bulls were evaluated before sampling in slaughterhouse, and those with a symptoms or history of gastrointestinal problems were excluded from the sampling process, and ileum tissue samples were prepared from slaughtered bulls between 2 and 4 years old from Urmia Industrial Slaughterhouse. The ileum was prepared less than 20 minutes after slaughtering, and then a complete 15 cm segment of ileum was cut, and a longitudinal incision then made to open the inner (mucosal) surface of the ileum. Tyrode's solution containing glucose (5.6 mM), NaCl (136.9mM), KCl (2.7 mM), $NaHCO_3$ (11.9mM), $MgCl_2$ (1.1 mM), $CaCl_2$ (1.8 mM), and NaH_2PO_4 (0.4 mM) was used as the rinse, transportation and incubation medium. The mucosal and serous surface of the ileum tissue samples were immediately rinsed with cooled (4 ˚C) and aerated Tyrode's solution to remove the digestive contents. Specimens were immersed in 4 °C Tyrode's solution and kept at this temperature until reaching the laboratory. The solution was exchanged 10 minutes after the initial collection to remove digestive contents and supply the

necessary material to the tissue. In the laboratory, whole ileum tissue pieces were placed on the dissected board filled with Tyrode's solution. The non-smooth muscle (mucosa and submucosa) was carefully separated from the ileum smooth muscle. The tissue strips (5 × 20 mm) cut in the path of the circular muscle layer (Jalilzadeh-Amin et al. 2012b).

Registration of smooth muscle activity. The ileum smooth muscle strips were housed in separate chambers, each containing 25 ML of Tyrode's solution at a temperature of 37 °C, and sparged without interruption with a mixture of 5% CO_2 and 95% O_2. For fixing the samples in chambers, one end of each tissue was connected to an isometric transducer with thread, and the other end was fixed to the down hook of the chambers (Model TRI 202p; PanLab, Barcelona, Spain). Six transducers linked to an amplifier (model ML224; AD Instruments, Castle Hill, Australia) and Power Lab data acquisition system (model ML870; AD Instruments) used for data collecting. Lab chart software (version 6.0, AD Instruments) was employed to view and record data.

Design of experiments. First of all, the ileum muscle samples were rested in the Tyrode's medium for one hour to reconcile to the novel environment. In this stage, the Tyrode solution was replaced every 15 minutes, and 2 one gr tensions applied to the tissues at 15-minute intervals. All specimens to evaluate the viability and contractile function tested before the main experiment by adding 10 µM of acetylcholine. After the acetylcholine, tissues were washed with Tyrode's solution to achieve basal contraction of muscles. This way was repeated three times and, if the results were the same, the tissue was considered acceptable for testing.

The effect of pulegone on the contractions of bovine ileum circular smooth muscle was investigated in four groups, each containing six tissue samples. In the first group, the effects of cumulative concentrations of pulegone were examined on the basal tonus. When the tissue samples reached a balance point in the tissue bath and followed a fixed baseline, the incremental concentrations of pulegone separate (1 to 1000 µg ml^{-1}) added bath cumulatively.

In the other groups, after the tissue samples reached equilibrium, they first underwent contraction under the influence of KCl (20 mM), Cch (1 µM), and $BaCl_2$ (3 mM), pulegone concentrations added to the organ bath and its cumulative effects on contractions were recorded.

Pulegone in separate dissolved in DMSO 5% and dilutions of 1, 3, 10, 30, 100, 300, and 1000 µg ml^{-1} prepared by adding the Tyrode solution. All concentrations of pulegone in separate groups were added to the medium at 2-minute intervals. In the end, the tissue samples were washed with Tyrode's solution. The viability of muscles confirmed by their response to the addition of 10 µM acetylcholine.

Statistical analysis. First of all, data graphically assessed using histogram and box plots, and also the assumption of data normality was tested by the Shapiro-Wilkes test. Since the assumptions required for the normal distribution and homogeneity of variances for parametric tests did not exist and different methods of changing the data did not work to meet these assumptions, the Nonparametric Friedman Repeated Measures Analysis of Variance on Ranks was employed to compare the results. Pairwise caparison between each concentration and the control were identified by the use of Dunnett's test. The significance level was set at $P< 0.05$. Results were expressed as medians and interquartile ranges (25th-75th percentiles). IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA) was used for statistical analysis of data.

Results

This study showed that different concentrations of pulegone significantly

decreased the basal and stimulated contractions in the bovine ileum smooth muscle. Cumulative concentrations of 30 to 1000 µg ml^{-1} pulegone caused a significant reduction in the spontaneous basal contraction of the ileum smooth muscle ($P<0.05$) (Fig 1, 2-A). Cch-induced contractions were significantly relaxed at 30 to 1000 µg ml^{-1} concentrations of pulegone ($P<0.05$) (Fig 1, 2-B). The spasmolytic effect on KCl (K20) induced contractions was significantly relaxed at 30 to 1000 µg ml^{-1} concentrations of pulegone ($P<0.05$) (Fig 1,2-C). $BaCl_2$-induced contractions were significantly inhibited at 30 to 1000 µg ml^{-1} concentration of pulegone ($P<0.05$) (Fig 1, 2-D). The spasmolytic effect on KCl (K60) induced contractions was significantly relaxed at 300 to 1000 µg ml^{-1} concentrations of pulegone ($P<0.05$) (Fig 2-E). The control treatments this study showed which the contractions created with the three spasmogens $BaCl_2$, KCl and Cch, survived for 15 minutes. The pulegone solvent (DMSO) did not affect ileum smooth muscle isolates' contraction. At all periods of the tests, the effects created by pulegone were eliminated after the tissue was rinsed. At the end of the test, the tissues showed a normal reaction to acetylcholine, indicating no damage to the tissue due to the presence of this substance.

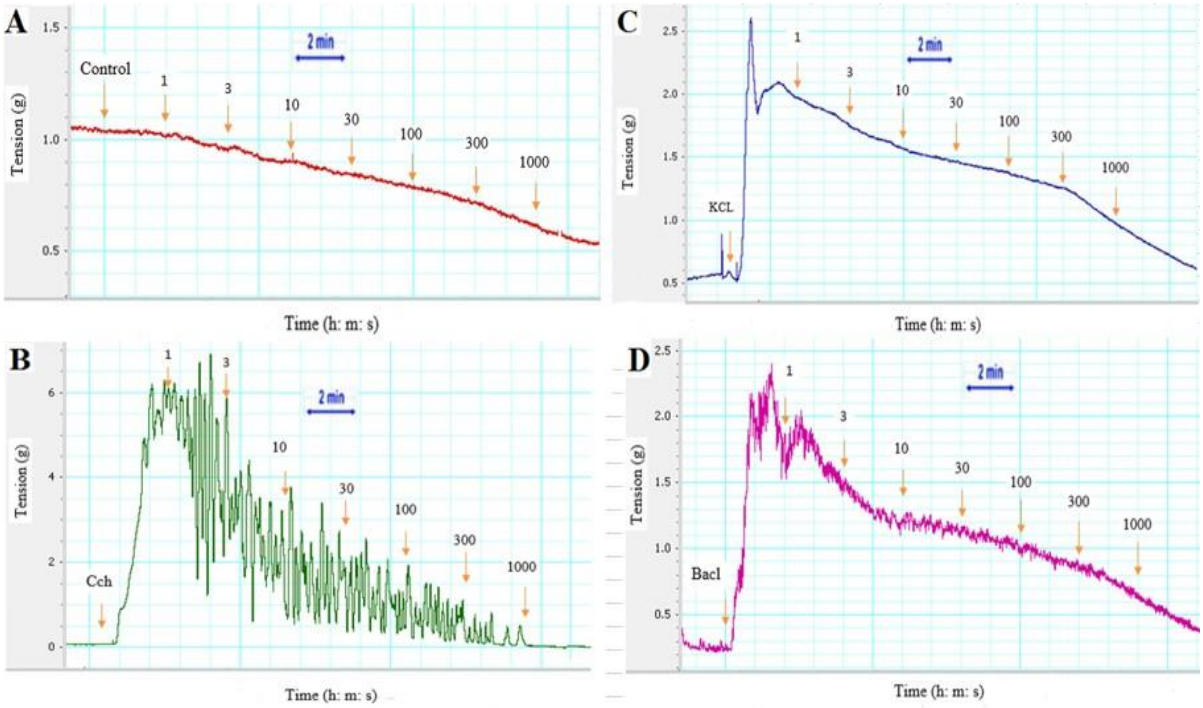
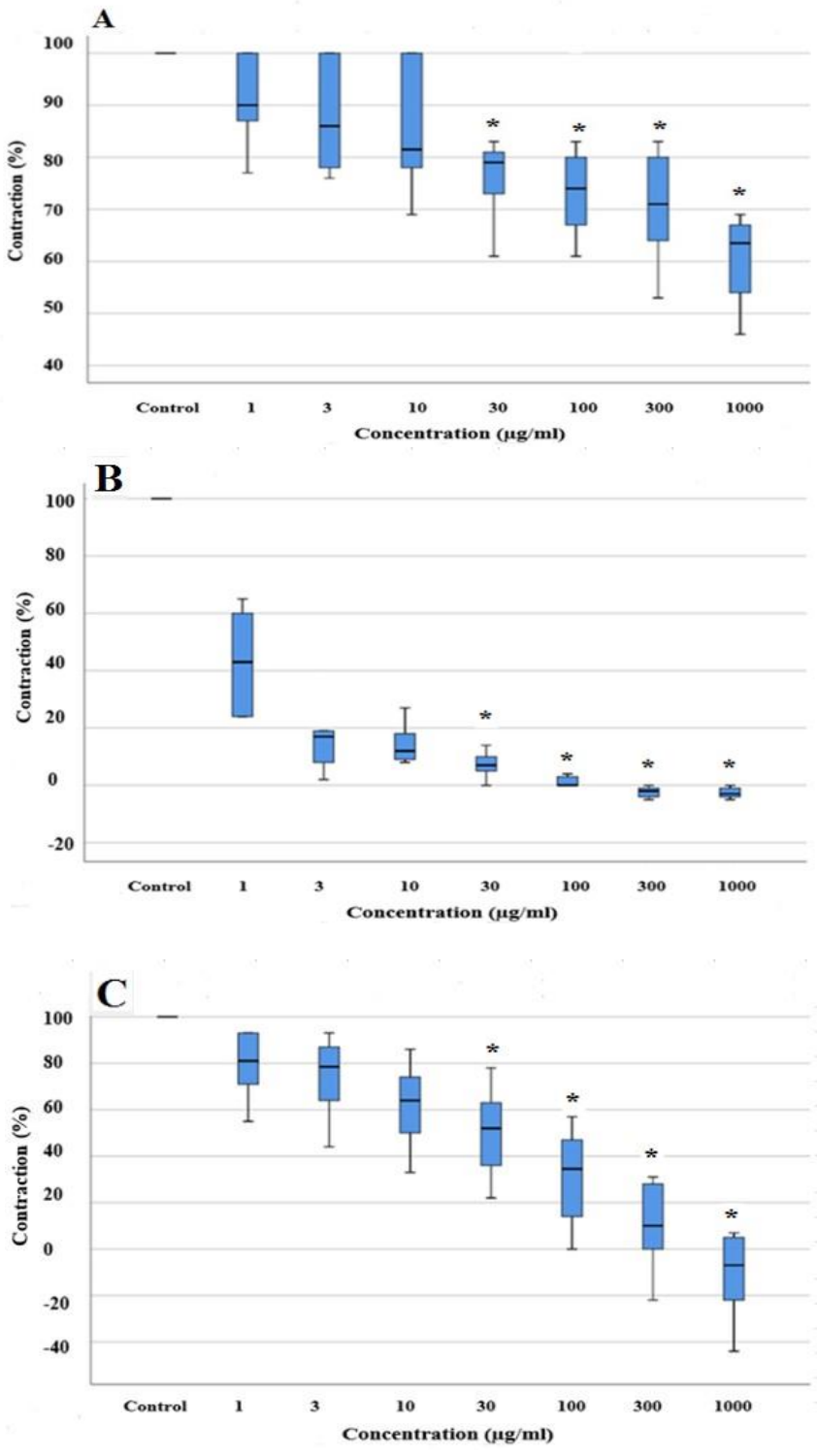


Fig. 1: The effect of different concentrations of pulegone on basal tonus (A); contraction induced by carbacol-induced (B) Potassium chloride (K20) (C) and barium chloride (D) contraction in the circular smooth muscles of bovine ileum.


A
Contraction (%)
100
90
80
70
60
50
40
Control
1
3
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30
100
300
1000
Concentration (µg/ml)
B
Contraction (%)
100
80
60
40
20
0
-20
Control
1
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Concentration (µg/ml)
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Contraction (%)
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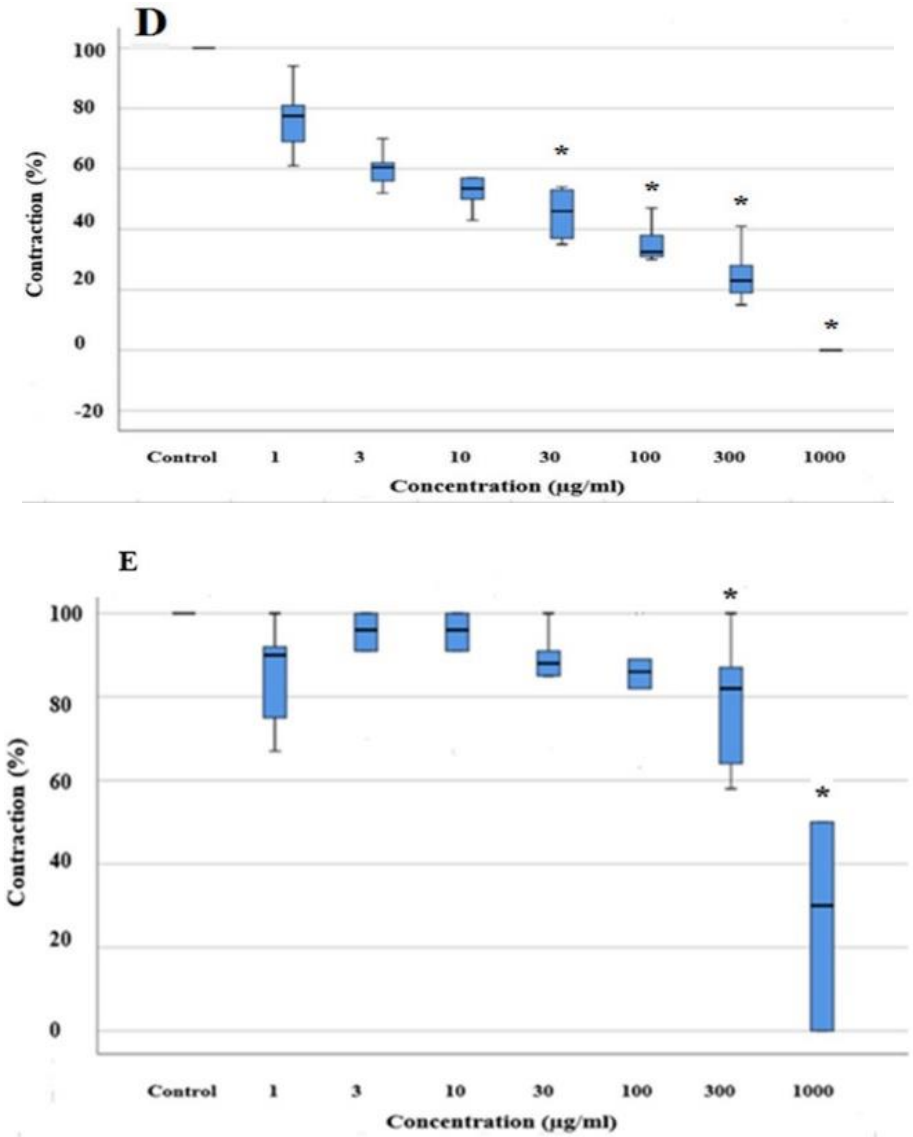

Fig. 2: The effect of different concentrations of pulegone on basal tonus (A); contraction induced by Carbacol-induced (B) Potassium chloride (K20) (C), Barium chloride (D) and Potassium chloride (K20) (E) contraction in the circular smooth muscles of bovine ileum. Results have been reported as the percentage of stable initial maximum contraction (SIMC) of each spasmogen and the percentage of stable initial basal contractions (SIBC) about spontaneous contractions. The star (*) indicates a significant difference compared to the control group (P< 0.05).

Discussion

This study investigated the effects of pulegone on contractions induced by several spasmogens in bovine ileum smooth muscle in an organ bath and the results revealed that pulegone significantly inhibits spontaneous contractions as well as all spasmogen-induced. Antispasmodics can block specific receptors or act in a non-specific way. Different pathways and receptors are involved in causing each of the spontaneous and induced (Cch, KCl, and $BaCl_2$) contractions (Souza et al. 2013).

This study has shown for the first time that pulegone relaxed tonic contractions induced by KCl (20), Cch, and $BaCl_2$ in the bovine smooth muscles of the ileum. These effects are coherent with pulegone's widespread use as an antispasmodic. Gastrointestinal motility disorders may be seen in two forms: decreased and increased

movement (Radostits et al. 2007). The increased gastrointestinal movements followed by the spasm of the smooth muscle of the cow intestine cause abdominal pain (Fecteau et al. 2017). Also, increased intestinal movements and reduced intestinal transfer time may lead to malabsorption and dyspepsia, and diarrhea due to insufficient time for digestion and absorption of consumed food (Heller and Chigerwe. 2018, Navarre and Roussel. 1996). Therefore, the use of a spasmolytic drug seems necessary in such cases to reduce gastrointestinal movements. Studies have shown that essential oils can decrease or increase the gastrointestinal movements of ruminants (Mendel et al .2017).

Study effects of *Mentha longifolia* essential oil on the abomasal and ruminal longitudinal smooth muscle in sheep suggested that this essential oil can manipulate abomasal and rumen contractions (Jalilzadeh-Amin et al. 2012). A study of the effects of essential oil of effects EOMP is likely to be mediated via inhibition of calcium entry, mainly by its primary compound, pulegone (Soares et al. 2012). *Mentha longifolia* is used as an antiemetic, particularly in chronic diarrhea and leaf extract this plant exerts relaxant effects on intestinal smooth muscle, consistent with the traditional use of the plant to treat gastrointestinal disorders such as colic and diarrhea (Mikaili et al. 2013). Pulegone exerts its negative inotropic effect on the guinea-pig and mouse heart (ventriculum or atria) mainly by the decrease of L-type Ca^{2+} current and the global intracellular Ca^{2+} transient (de Cerqueira et al. 2011).

Evaluation of pulegone on transit time and castor-oil induced diarrhea in rats shown pulegone at the dose of 25 mg/kg significantly reduced the volume of intestinal secretion induced by castor oil (Jalilzadeh and Maham. 2013). Monoterpene compounds, similar to pulegone, have also had their inhibitory effects reported. Pinocamphinone and isopinocamphone inhibited the contractions elicited by and $Bacl_2$ and Ach (de Cerqueira et al. 2011, Soares et al. 2012). One study indicates peppermint oil exerts $Ca2^{+}$ channel blocking properties that may underlie their use in irritable bowel syndrome (Hawthorn et al. 1988). Study the vasorelaxant activity of monoterpenes showed that they have vasorelaxant potency and efficacy (Lima et al. 2012). To the best of our knowledge, our study reveals the first record of pulegone impact on bovine ileum contractility.

In conclusion, pulegone with reducing spontaneous and induced contractions in-vitro on the bovine ileum reveals its ability to relieve spasms and control intestinal hypermotility in bovine. Therefore, pulegone may be used to treat ruminant gastrointestinal disorders for example the intestinal hypermotility or acute abdomen. However, further research should be done to evaluate the effects of pulegone on gastrointestinal motility in-vivo.

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Conflict of interest

The authors declare no competing financial interest.

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فعالیت اسپاسمولیتیک پولگون بر انقباضات ایلئوم گاو

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چکیده

اختلالات حرکتی دستگاه گوارش در نشخوارکنندگان با اهمیت است. پولگون یک کتون مونوترپن طبیعی است که از اسانس گیاهان مختلف به دست می‌آید. گزارش شده که پولگون دارای اثرات ضد باکتریایی، ضد قارچی و ضد هیستامین است. مطالعه حاضر اثرات پولگون را بر انقباضات عضله صاف ایلئوم گاوی بررسی کرده است. این آزمایش بر روی عضله صاف بافت ایلئوم تهیه شده از کشتارگاه در حمام بافتی انجام شد. هفت غلظت تجمعی پولگون از ۱ تا ۱۰۰۰ میکروگرم در میلی‌لیتر به نمونه‌های بافت اضافه شد. محلول مورد استفاده محلول تیرود بود که با مخلوطی از ۹۵٪ اکسیژن و ۵٪ دی اکسید کربن هوادهی می‌شد و دما در ۳۷ درجه سانتی‌گراد تنظیم شد. اثر پولگون بر انقباضات پایه و سه انقباض ایجاد شده ناشی از باریم‌کلراید، پتاسیم‌کلراید و کارباکول بررسی شده است. نتایج نشان داد که پولگون به طور قابل توجهی انقباضات خود به خودی و همچنین انقباضات ناشی از اسپاسموژن‌ها مهار می‌کند. پولگون انقباضات ناشی از کارباکول، باریم‌کلراید و پتاسیم‌کلراید را در غلظت ۳۰ میکروگرم بر میلی‌لیتر شل کرد. پولگون دارای اثر اسپاسمولیتیک می‌باشد و این می‌تواند اساس فارماکولوژیکی برای استفاده‌ی دارویی در درمان اسپاسم روده یا افزایش حرکات باشد.

کلمات کلیدی: اسپاسمولیتیک، پولگون، ایلئوم، گاو

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