



# Evaluation of Pulegone on Transit Time and Castor-Oil Induced Diarrhea in Rat

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## ABSTRACT

**Background:** Pulegone is an allelochemical widely occurring in plants of the Labiatae family. Pulegone has a pleasant mint like odor and therefore is used, directly or as a constituent of a variety of essential oils, in beverages and processed foods for human consumption. **Methods:** To evaluate the antidiarrheal activity of pulegone using various pharmacological models, the intestinal transit, castor oil induced diarrhea and enteropooling methods were used in this study. The acute toxicity and lethality of pulegone was determined using the Lorke's method. **Results:** The pulegone was practically nontoxic administered p.o. The LD<sub>50</sub> was 570 mg/kg given p.o. Pulegone (25–100 mg/kg, p.o.) produced insignificant reduction in propulsive movement in the normal and significant ( $P < 0.05$ ) reduction in castor oil induced intestinal transit tests in rats. Peak effect was elicited at 25 mg/kg but this effect was higher than that produced by loperamide (3 mg/kg, p.o.). In the castor oil induced diarrhea test, high dose of pulegone significantly delayed the onset but did not decrease the frequency and severity of diarrhea. Pulegone at the dose of 25 mg/kg significantly reduced the volume of intestinal secretion induced by castor oil but produced no effect on diarrhea parameters. **Conclusion:** The results obtained in this study suggest that the pulegone did not possess antidiarrheal property due to weak inhibition of gastrointestinal propulsion and fluid secretion.

## Introduction

Diarrhea syndrome is characterized by discharge of semisolid or watery faecal matter three or more times in a day. It involves an increase in the fluidity, volume and frequency of intestinal movements. Clinically it increase bowel sound frequency, wet stools defecation and abdominal pain, consequently increased secretion and decreased absorption of fluid that lead to loss of water and electrolytes.<sup>1</sup> Although advances in understanding of the causes, treatment and management of diarrheal diseases were progressed, in developing countries many people especially including 2.5 million children die from diarrhea every year.<sup>2</sup> Rehydration therapy remains the most important treatment, although it does not reduce the severity of diarrhea. Other treatment options include antibiotics and gut motility suppressing agents, which aim to reverse dehydration and shorten the length of illness.<sup>2</sup>

There have been numerous reports of the use of traditional plants for the treatment of diarrheal diseases. *Mentha longifolia* (Lamiaceae) is an aromatic herb (locally called "Nana" in Urmia, Iran), was grown commercially for production of oils or leaves because for centuries this plant has been chiefly used traditionally in folk remedies for valuable effects on the digestion and gastrointestinal disorders.<sup>3</sup>

The chemical composition of its essential oil has been determined by GC/MS and the main reported compounds were 1,8-cineole (19.8%), pulegone (17.3%), caryophyllene oxide (14.8%) and isomenthone

(12.4%).<sup>4</sup> In earlier work we evaluated the spasmolytic activity of this plant on smooth muscles contractile response and found that the essential oil is responsible for this activity.<sup>4</sup>

Whereas the natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds and many of which have been the basis for the development of new lead chemicals for pharmaceuticals. At this time we decided to study main compounds identified in the essential oil in order to recognize which compound(s) are responsible for biological activity. This study was carried out to investigate pulegone role.

Pulegone is natural monoterpene ketone (Terpenoid) obtained from the essential oil of a variety of plants such as *Nepeta cataria* (Catnip) and *Mentha* species. *M. pulegium* L. contains 60-90%<sup>5</sup> and *Mentha longifolia* L. contains 17%<sup>4</sup> pulegone as principal component of essential oil, reported to have anti bacterial, anti-feedent, pesticidal and insect repellent properties.<sup>6</sup> Commercially, it was used as flavoring agent for toothpastes and mouthwashes, as valuable ingredient for perfumes and various pharmaceuticals and has been utilized in aromatherapy.<sup>6</sup> The herbs with high pulegone content have been used as components of herbal teas for stomach disorders in Turkey.<sup>7</sup> Experimentally, antihistaminic properties and inhibitory effect on the contractile activity of the isolated intestine<sup>8</sup> and myometrium<sup>7</sup> were demonstrated

for pulegone. Guided by ethnobotanical literature and availability from natural sources and following in vitro studies<sup>7-9</sup> our main object was to assess the effects of the pulegone, the principal component in the many of medicinal plants oil, on the intestinal transit and diarrhea induced with castor oil.

## Materials and Methods

### Animals

Wistar rats (210–230 g) of either sex obtained from the central animal house of this University were used. The animals were fed with standard pellet diet and water *ad libitum*. Food was withheld 24 h prior to experimentation but they were allowed free access to water. Groups of six rats were included in the study. All experiments were approved by the Animal Care and Use Committee at the Urmia University and are in accordance with the guidelines for Care and Use of Laboratory Animals.

### Acute oral toxicity and LD50 determination test

Acute toxicity tests were performed in both male and female rats in order to evaluate the toxic effect of pulegone (10, 100, 1000, 1600, 2900 and 5000 mg/kg, p.o.) in different gender groups. The general signs of toxicity (i.e., convulsions, ataxia, hypoactivity, ventilation disorders) and mortality rate were recorded hourly in first day and subsequently all rats were observed twice daily for 14 days after dosing, and then killed and necropsied. The LD<sub>50</sub> for the pulegone were determined using the method described by Lorke.<sup>10</sup>

### Normal Small intestine transit

The effect of the pulegone on small intestinal transit was studied with seven groups containing six animals in each group. These groups were control (%2 of Tween 80); 25, 50, 75, and 100 mg/kg of the pulegone; 0.1mg/kg atropine sulphate in oral route. Seventh group received atropine sulphate thirty minutes before pulegone (75mg/kg) administration. Thirty minutes after treatment, rats were given 2 ml charcoal meals (5% charcoal in 5% tragacanth) by oral route. All animals were scarified after 30 min; movements of charcoal from pylorus to caecum were measured and the results were expressed as a percentage distance travelled compared with the negative control. Peristaltic index for each mouse was then expressed as a percentage of the distance traveled by the charcoal meal relative to the total length of the small intestinal.<sup>11</sup>

### Castor oil induced small intestinal transit

Castor oil (2 mL/rat, p.o.) was administered to groups of rats that received 30 min before, the pulegone (25 and 100 mg/kg), loperamide (3 mg/kg) and vehicle by oral route. Thirty minutes later, each animal was given 1 mL of a charcoal marker (10% suspension in 5% tragacanth powder). After 20 min, the rats were killed and the distance travelled by the marker was measured and expressed as a percent of the total length of intestine from the pylorus to caecum.<sup>12</sup>

### Castor oil induced diarrhea

The method of Atta and Mouneir<sup>13</sup> was applied on four

groups of animals. Group 1 was given 2% Tween 80 solution and kept as a control, while group 2 was given loperamide (3 mg/kg) as a standard antidiarrheal drug. The rats of groups 3 and 4 were given pulegone orally in a dose of 25 and 100 mg/kg. One hour after administration of pulegone, all animals received 2 mL of castor oil orally. The animals were then placed under separate cages, with the floor lined with paper, for observation for 4h. The following parameters were observed: the time delayed between the administration of castor oil and the excretion of the first diarrheic faeces, the total number of faecal output and the number of diarrheic stools excreted by the animals in 4 h, as well as the total weight of the diarrheic faeces in that period of time. The severity of the castor oil-induced diarrhea was noted and recorded as a score. A numerical score based on stool consistency, was assigned as follows: normal stool (or lack of diarrhea), 1; semi-solid stool, 2; watery stool/faeces, 3, respectively.

### Intestinal fluid accumulation (enteropooling assay)

Following the method of Adeyemi et al.<sup>14</sup>, rats divided into groups were pretreated with distilled water (10ml/kg, p.o.), loperamide (3 mg/kg) and pulegone (25 and 100 mg/kg, p.o.). One hour after, the rats received castor oil (2 ml/rat) intragastrically. The animals were killed 1 h later and the small intestines were removed after ligation at the pyloric end and ileocaecal junction, respectively. The contents of the intestine were then expelled into a graduated tube and the volume measured.

### Drugs/chemicals

Pulegone, Loperamide (standard reference antidiarrheic drug) and atropine sulphate, were purchased from Sigma Chemical Co., USA, castor oil (laxative agent), Tween 80 obtained from Merk (Germany). For the experiments, the pulegone were dissolved in 2% Tween, charcoal meal, atropine sulphate and loperamide were prepared in normal saline.

### Statistical Analysis

Data are presented as means  $\pm$  S.E.mean. Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the control group. A *P* values < 0.05 were considered significant.

## Results

### Acute toxicity test

Oral administration of the pulegone in doses 10 and 100 g/kg did not produce any mortality and visible signs of toxicity when observed 24 h after treatment and for further 14 days. Administered orally, the pulegone produced visible signs of toxicity in rat at doses of greater than 100 mg/kg. These include abnormal gait, increased respiration, decreased activity, unresponsive to writhing test, and limb paralysis. The LD<sub>50</sub> was estimated to be 570 mg/kg (3.74 mmol/kg)

for the oral route. Visual inspection, on necropsy, did not reveal any signs of damage to organs.

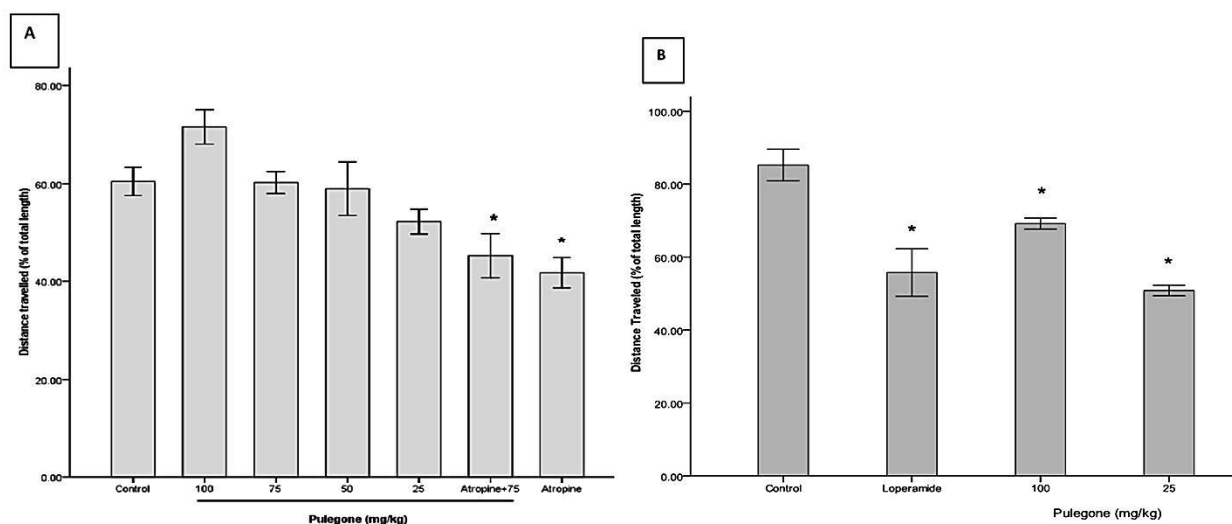
#### Normal Small intestine transit by charcoal meal test

Pulegone was tested on gastrointestinal motility by charcoal meal test. Insignificant increase in the intestinal motility was observed in 100 mg/kg of pulegone. Under similar experimental conditions the percentage decrease in intestinal transit time caused by Atropine sulphate was 41.75% at a dose 10mg/kg. The results shown that pulegone at doses of 75, 50 and 25 mg/kg caused insubstantial decrease in intestinal transit time by 60.25%, 59% and 52.25% respectively compared to control. In contrast, a high dose of

pulegone 75 mg/kg with atropine significantly decreases the marker transit (Figure.1.A).

#### Effect on Castor oil induced small intestinal transit

Administration of castor oil elicited an amplification of intestinal transit, rising the distance travelled by the marker from 60.50% to 85.05% in control group when tow methods were compared together (Figure.1.A and B). Additional application of pulegone at doses of 100 and 25 mg/kg and reference drug loperamide (3 mg/kg) produced significant inhibitions on castor oil induced small intestinal transit (Figure.1.B). The effect observed with pulegone was dose-dependent.



**Fig.1.** Effects of the pulegone on rat intestinal transit time. Doses were used including; pulegone (25, 50, 75 and 100 mg/kg), Atropine (0.1 mg/kg) and Loperamide (3mg/kg). Graphs showing the action of pulegone on intestinal transit time, expressed as %distance travelled down the intestine, in (A) control animals and (B) those receiving castor oil pretreatment (CO; 10 mg/kg body weight, 30 min before test meal). (\* indicates values significantly different from 'control' in both graphs;  $p < 0.05$ , Dunnet test).

#### Effect on castor oil induced diarrhea

In controls, copious diarrhea was evident in 100% of rats in the second hour following the oral administration of castor oil. Oral administration of loperamide in a dose of 3 mg/kg induced a significant ( $p < 0.05$ , 0.01) antidiarrheal effect against castor oil-induced diarrhea for up to 4 h post-administration. Only in the first hour after diarrhea induction, pulegone at 100 and 25 mg/kg has significant protection against it (Table.1). However pulegone did not show considerable activity in delaying in onset of diarrhea, frequency of stooling (reduction in number of wet stools and total stools), decrease in weight of wet stools and the general diarrhea score including the hard, mild and copious diarrhea (Table. 2).

Pretreatment of rats with the pulegone 100 mg/kg caused a significant ( $p < 0.05$ ) delay in onset of diarrhea. The standard anti-diarrheal drug, loperamide (3 mg/kg, p.o.) produced a significantly ( $p < 0.05$ ) greater inhibitory effects on all the diarrhea parameters investigated than the highest dose of the pulegone (Table. 2).

#### Effect on intestinal fluid accumulation (enteropooling assay)

The castor oil induced intraluminal accumulation of fluid is inhibited by 52% at 25 mg/kg pulegone. The reference drug loperamide at a dose of 3 mg/kg reduced intestinal secretion by 43.69%. Both these values were significant as compared with control, as shown in Table 3.

#### Discussion

Diarrhea is a public problem, especially among children and contributes too much morbidity and mortality. Diarrhea may result from disturbed bowel function in which case there is impaired intestinal absorption, excessive intestinal secretion of water and electrolytes and a rapid bowel transit. Manonmani *et al.*<sup>15</sup> have shown that prior treatment with some plant extracts had a protective effect on the intestinal tract. Pulegone is a major constituent of several essential oils (e.g., peppermint, pennyroyal) used for flavoring foods and drinks. One of them is *Mentha longifolia* essential oils, which has been reported to contain 17% pulegone, has also been used as a fragrance agent and as an herbal medicine to treatment of gastrointestinal disorders that rationalized with *in vitro* study.<sup>4</sup> However there is no study available showing pulegone efficacy in hypo- and

hyperactive gut disorders, such as colic and diarrhea. This study attempted to provide a basis for its

medicinal use of pulegone rich herbs in these conditions, which have not been studied so far.

**Table 1.** Acute oral toxicity of pulegone in rats. The pulegone, dissolved in Tween 80 (2%) was administered as single oral doses to 6 groups of 5 rats. All animals were carefully examined for adverse effects (behavioural changes and mortality) for 14 days. Symptoms of toxicity are described for a group; D/T: dead/treated rats; latency: time to death after the dose; none: no toxic symptom during the observation period.

Treatment (mg/kg)	D/T	Effects	
		Mortality latency (h)	Symptoms of toxicity
10	0/5	-	None
100	0/5	-	None
400	0/5	-	Ataxia, Hypoactivity
800	5/5	>3, <4	Ataxia, Hypoactivity, flaccid paralysis, recumbency
1000	4/5	>2, <4	Ataxia, Hypoactivity, flaccid paralysis, recumbency
1600	4/5	>2, <3	Ataxia, Hypoactivity, flaccid paralysis, paddling, recumbency

**Table 2.** Effect of pulegone on the time course of diarrhea induced by castor oil in rats. Values are expressed as the percentage of animal with diarrhea (n=6). <sup>a</sup> p< 0.05 vs vehicle control (Chi-square test).

Treatment (mg/kg)	Animals with diarrhea (%)			
	1 h	2 h	3 h	4 h
Vehicle control (Tween 2%)	0	100	100	100
Pulegone (100)	0	0 <sup>a</sup>	100	83.33
Pulegone (25)	0	66.66 <sup>a</sup>	83.33	83.33
Loperamide (3)	0	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>

**Table 3.** Antidiarrheal activity of pulegone on the diarrhea parameters that induced by castor oil in rats.

Treatment (mg/kg)	Onset time of diarrhea (min.)	1 h			2 h			3 h			4 h		
		F.NO*	F.W*	D.S*	F.NO	F.W	D.S	F.NO	F.W	D.S	F.NO	F.W	D.S
Vehicle control (Tween 2%)	103.66±20.73	0.00	0.00	0.00	1.50±0.61	1.21±0.50	4.83±1.93	1.66±0.21	1.28±0.08	3.33±0.33	2.16±0.16	1.10±0.03	4.33±0.61
Pulegone (100)	137.83±11.63 <sup>a</sup>	0.00	0.00	0.00	0.00	0.00 <sup>a</sup>	0.00	3.66±0.76	1.33±0.13	7.16±1.10	3.50±0.84	1.51±0.41	6.66±1.54
Pulegone (25)	123.16±14.59	0.00	0.00	0.00	2.16±0.70	0.90±0.29	6.16±2.12	3.50±0.76 <sup>a</sup>	1.86±0.38	7.33±1.66	3.33±0.88	1.33±0.29	6.50±1.40
Loperamide (3)	>240 <sup>a</sup>	0.00	0.00	0.00	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>

**Table 4.** Effect of pulegone on castor oil-induced enteropooling in rat. Values are expressed as mean±S.E (n=6). <sup>a</sup> p< 0.05 vs vehicle control.

Treatment (mg/kg)	Fluid volume (ml)	% inhibition
Vehicle control (Tween 2%)	3.25± 0.28	-
Pulegone (100)	3.30± 0.13	1.53
Pulegone (25)	1.56± 0.26	52*
Loperamide (3)	1.83 ± 0.16	43.69*

Acute lethal doses are available for pulegone. An LD<sub>50</sub> of 1709 mg/kg (11.23 mmol/kg) was reported for mice when it given by the subcutaneous route, while rats exposed by the intraperitoneal and oral routes had an LD<sub>50</sub> of 150 mg/kg (0.985 mmol/kg) and 470 mg/kg (3.08 mmol/kg) respectively.<sup>16</sup> According to our findings, LD<sub>50</sub> is 570 mg/kg (3.74 mmol/kg) was in much agreement with previous report. Acute oral toxicity studies in rats indicate that pulegone is not associated with any untoward toxicological effects at doses of 10 and 100 mg/kg. Administration of R (+)-pulegone at a dose of 100 mg/kg bw per day caused an increase in alanine aminotransferase activity, but no other effects were seen.<sup>17</sup> Thus, it is relatively safe through the oral route. For this reason dose 100 mg/kg was selected as highest treatment dose. To date although the mechanism by which it cause acute lethal cell injury fully revealed, little is known about the clinical sings of acute pulegone-mediated toxicity. Clinical sing actually followed in this study, include abnormal gait, increased respiration, decreased activity, unresponsive to writhing test, and limb paralysis.

The pulegone produced an insignificant increase and decrease in propulsive movement at the standard charcoal meal in the small intestine, suggesting a weak spasmolytic activity. This activity was reverse dose dependent with the greatest antispasmodic effect shown at 25 mg/kg of the pulegone. It seems that two types of receptors therefore appeared to be involved in pulegone action on gastrointestinal tract: high and low affinity receptor subtypes sensitive to low and high agonist concentrations, which induced contraction and relaxation of smooth muscles, respectively.<sup>4</sup> The pulegone was more effective in the castor oil induced intestinal transit than in the normal transit, this result suggested that the pulegone may be more effective in an altered state than in normal state.

The castor oil test has been extensively used as a basic pharmacological test to study the role of endogenous substances involved in diarrhea and to screen antidiarrheal drugs. This test is characterized by the reproducible evacuation of watery stools two hours after castor oil administration.<sup>18</sup> Castor oil causes diarrhea through its active metabolite ricinolic acid<sup>19</sup>, which stimulates the peristaltic activity of small intestine leading to changes in electrolyte permeability of intestinal mucosa. Its action is also associated with stimulation of release of endogenous prostaglandins.<sup>20</sup> In the antidiarrheal study, the pulegone exhibited insignificant inhibitory activity against castor oil-induced diarrhea at all doses. The results were dose-dependent and comparable with that of standard drug as loperamide.

Pulegone had significantly reduced intestinal motility as observed by the decrease in intestinal transit in rats pretreated with castor oil. The reduction in the intestinal motility may be responsible for the antidiarrheal activity. Probably pulegone at low doses increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit

by charcoal meal and by their anticholinergic and antihistaminic effects.<sup>8,9</sup>

In the enteropooling assay the maximal effect of the pulegon was similar to loperamide, which is one of the most efficacious and widely used antidiarrheal drugs; as show in present study loperamide effectively antagonized diarrhea induced by castor oil.<sup>21</sup> The therapeutic effect of loperamide is believed to be due to its anti-motility and anti-secretory properties. The mechanisms of anti-secretory action of loperamide have been discussed with reference to: 1) opiate agonism, 2) block of calcium channels, and 3) inhibition of calmodulin.<sup>22</sup> From the study, it is likely that pulegone may mediate its effects through similar mechanisms. It is well known that some terpenoides can act as spasmolytic agents by involving calcium antagonism.<sup>23</sup> Similarly pulegone, a terpenoide, inhibited spontaneous and agonist induced contractions of the rat illeum<sup>9</sup> and we show slightly reduced the intestinal transit rate. It has been demonstrated that pulegone inhibit contractions induced by spasmogens in smooth muscle isolated from rat and guinea-pig intestine<sup>8,9</sup> and myometrium<sup>7</sup> and we have revealed, for the first time, it's in vivo pharmacological activity by showing inhibitory effects on small intestinal transit that is in conformity with others findings. According the results pulegone has weak anti-motility and very efficacious anti-secretory activity. Overall these effects collectively may contribute to the appearance of no antidiarrheal activity. An antimicrobial activity of the pulegone against common pathogens involved in gastroenteritis has been reported<sup>24</sup> and is likely to contribute to the antidiarrheal potency during infectious diarrhea.

The WHO criteria for the acceptance of a drug as an antidiarrheal include: (1) inhibition of the production of wet or unformed faeces in animals; (2) inhibition of the production of watery stool or fluid evacuation in animal and (3) inhibition of gastrointestinal propulsive action.<sup>25</sup> The pulegone therefore, don't meet any of these criteria as observed from its results. This study did not support the use of pulegone as an antidiarrheal agent. Although medicinal plants with high percent of pulegone has been used orally as a remedy for the treatment of gastrointestinal disturbances and some of them are consumed as a vegetable every day.

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