The Laxative and Prokinetic Effects of *Rosa damascena* Mill in Rats

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**Abstract**

**Objective(s)**  
This study was aimed to assess the possible laxative and prokinetic effects of the boiled extract of *Rosa damascena*.

**Materials and Methods**  
Rats in two groups (n= 7) of test and control were gavaged either with the extract or placebo, respectively. The number, weight and water percentage of feces were studied up to 24 hr. In order to assess the possible osmotic laxative effects of the drug, the jejunum in anesthetized rats (n= 7) was randomly divided into 4 cm segments and 0.5 ml of the extract, lactulose or saline was injected in each segment. The volumes of the contents in each segment were measured after 1 hr. In order to assess the intestinal transit time, fasting rats were gavaged with either the extract or placebo. Thirty minutes following the last medication, all rats were gavaged with phenol red and methyl cellulose (1.5 ml). The test and the control rats, in groups of 4, were sacrificed at 30 min, 1, 2 and 4 hr, and the amounts of the phenol red in various parts of the gastrointestinal tract were measured.

**Results**  
Boiled extract of *R. damascena* significantly increased feces number and its percentage of water, but had no effects on the transit time of intestinal ingesta. The volume of the contents in jejunum segments had significantly increased with the extract or lactulose compared to placebo.

**Conclusion**  
Boiled extract of *R. damascena* apparently exerts its laxative effects, at least in part, via osmotic infiltration of fluids into the intestine.

**Keywords:** Laxative, Prokinetic, *Rosa damascena*

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Introduction
Rosa damascena Mill. is especially important in production of rose oil, a key component in natural fragrance industry (1). However, this plant is also important for its various therapeutic effects in traditional medicine. For instance, it is recommended for treatment of abdominal chest pain, strengthening the heart (2), treatment of menstrual bleeding and digestive disorders (3) in ancient medical books. It has also been traditionally used for its analgesic, antidepressant, anti-inflammatory, diuretic and its mild laxative effects (4). There is not sufficient scientific evidence to confirm these effects. However, according to the recent studies, R. damascena seems to have strong antibacterial effects against a wide variety of bacteria including Aeromnas, Bacillus, Enterobacter, Enterococcus, Escherichia, Klebsiella, Mycobacterium, Proteus, Pseudomonas, Salmonella, Staphylococcus and Yersinia spp. (5-7). In addition, strong antioxidant properties have been shown for the plant (6, 8). Furthermore, moderate anti HIV activity has been reported for water and methanol extract of the plant in recent studies (9). A wide range of other therapeutic effects including antitussive (10), relaxant (11), anticonvulsant (12, 13), antidiabetic (14) and analgesic (15) effects have been also described for the petals of R. damascena in recent years.

According to the available databases, to the best of our knowledge, there is no scientific report regarding the laxative effects of R. damascena. The current research was aimed to verify the possible prokinetic and laxative effects of the plant in rats.

Materials and Methods
Wistar rats of either sexes weighing 180-230 g (Razi Vaccine and Serum Research Institute, Mashhad, Iran) were used. The animals were housed and allowed to acclimate to standard environment conditions in the animal unit of the School of Veterinary Medicine, Ferdowsi University of Mashhad at least for one week before the experiment.

Dried flower petals of R. damascena were purchased from the market and the genus and the species were confirmed by the Research Center for Plant Sciences, Ferdowsi University of Mashhad (Herbarium no: 10972, FUMH). The petals were boiled in distilled water (33 g/l) for 10 min. The filtrate was evaporated at 4 °C to achieve a final concentration of 1.5 g/ml. The dry matter of the extract was calculated to be 0.52 of the original flower petals. Therefore, the used dose (1.5 g/kg) equaled 0.78 g/kg in terms of the dry matter of the extract.

The laxative effects
The laxative effects of R. damascena was studied using the extract and the control groups (7 rats for each group). The rats in the test group were gavaged with the boiled extract of the plant at 1.5 g/kg Bwt, while the control rats received placebo. The excreted feces were counted up to 32 hr. In order to measure the water content (16-18), fecal samples were also collected every 15 min up to 16 hr. To achieve this, the samples were weighed both immediately and after drying (50 °C, 18-20 hr).

In order to assess the possible effects of the plant extract on intestinal secretions or osmotic infiltration of fluids into the gut lumen, 7 new rats were anesthetized with pentobarbital sodium (60 mg/kg), the abdominal cavity was opened, and the jejunum was randomly divided into three segments of 4 cm. Within each segment, the boiled extract of R. damascena (1.5 g/ml), lactulose (as positive control; 0.33 g/ml) or placebo (as negative control) were injected (0.5 ml), in a random order. One hour later, the volume of the fluid in each segment was measured.

In order to rule out the possible stimulatory effect of the extract on intestinal secretions, in a separate experiment, 6 rats were divided in 3 groups of 2. The first group received the plant extract (1.5 g/kg) by gavage, the second group received the same medication via intraperitoneal (i.p.) injection. The third group was the same as the first one but received placebo. Following the next 24 hours, the average feces numbers were counted in each group.

Intestinal transit time
The possible effect of R. damascena on intestinal transit time was studied using phenol red as previously described (19-21) with slight modifications. Briefly, 32 rats were randomly...

designated to the control and the test groups (4 groups of 4, each). During the experiment, the rats were deprived from food but had access to water. The test animals were gavaged with the boiled extract (1.5 g/kg Bwt) twice, with 18 hr intervals. The control rats received similar volumes of placebo (distilled water). Thirty minutes following the last medication, all rats in both control and test groups were euthanized in groups of 4 following 30 min, 1, 2 and 4 hr in a CO₂ chamber. Two additional rats were considered as the blanks and were euthanized immediately following administration of phenol red and methyl cellulose.

The abdominal cavity was opened; the small intestine was divided into 3 equal segments (S1-S3) and was carefully removed. The cecum and the colon were also removed. Each segment was separately washed with 0.9% saline and was homogenized within 100 ml NaOH 0.1 N solution. The suspension was allowed to settle at room temperature for 1 hr, and then 5 ml of the supernatant was added to 0.5 ml 20% trichloroacetic acid and centrifuged at 3000 rpm at 4 °C for 30 min. The supernatant was added to 4 ml 0.5 N NaOH, stirred and the absorbance of the sample was read at 560 nm (Jenway, UK). The absorbance of each sample was subtracted from the mean absorbance of the blanks. The concentration of phenol red was then calculated according to a calibration curve. Intestinal transit was determined by measuring the partitioning of dye within the small bowel segments and colon: numbered 1-5, proximal to distal. The geometric center of dye transit was calculated for each animal as (Σ (% dye per segment × segment number) /100).

Statistics
Statistical analysis and drawing of the figures were performed using GraphPad Prism v4.0 (GraphPad Software, USA). Statistical comparisons were performed using t-test for feces count and feces water percentage; two-way analysis of variance (ANOVA) followed by Bonferroni posttests for cumulative phenol red and geometric centre; and one-way ANOVA followed by Dunnett's test for osmotic infiltration of fluids into the jejunum. In all cases, P< 0.05 was considered as significant. Unless otherwise mentioned, all data are represented as mean±SEM.

Results
The laxative effect
The laxative effect of R. damascena was studied using feces count and feces water content. The average feces counts were significantly higher (P= 0.019) in the test group compared to those of the control during 32 hr of the experiment (Figure 1). The mean percentage of feces water was significantly higher (P= 0.035) in treated animals compared to the control group (65.8+2.4% vs 57.5+2.2; Figure 2). Most rats (57%) in the test group had watery feces during hours 16-24 of the experiment on visual examination.

The jejunum segments filled with R. damascena extract had significantly higher (more than 4 times; P< 0.0001) volumes compared to those filled with placebo (Figure 3). This was consistent to the results obtained with lactulose, used as the positive control.

In the complementary study regarding the possible stimulatory effect of the extract on intestinal secretions, the rats received the extract by gavage had an average feces number of 43, compared to 35 in those received placebo; while the rats received the extract via i.p. injection showed no defecation during 24 hr of the experiment.

![Figure 1. The feces counts in the test group up to 32 hr following intragastric administration of the boiled extract of Rosa damascena (1.5 g/kg Bwt, n= 7) compared to the control (n= 7). Data are represented as mean±SEM (*P = 0.019).](https://www.SID.ir)
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Figure 2. The percentage of feces water content in the test group up to 16 hr following intragastric administration of the boiled extract of Rosa damascena (1.5 g/kg Bwt, n= 7) compared to the control (n= 7). Data are represented as mean± SEM (*P= 0.035).

Figure 3. The fluid volumes within jejunum segments (n= 7) filled with Rosa damascena boiled extract (1.5 g/ml) compared to those filled with placebo (saline) or lactulose (0.33 g/ml). Data are represented as mean±SEM (**P< 0.0001).

Figure 4. Intestinal transit time, assessed using cumulative concentration of phenol red within the intestine, in rats gavaged with the boiled extract of Rosa damascena (1.5 g/kg Bwt twice with 18 hr interval) compared to that of the control group. Data in each time-point represent replicates of 4. Figures A-D stand for intestinal transit times at 30 min 1 hr, 2 hr and 4 hr respectively. Data are represented as mean±SEM (P> 0.05).
Figure 5. The geometric center of phenol red within the intestine in rats receiving the boiled extract of *Rosa damascena* (1.5 g/kg Bwt twice with 18 hr interval) compared to that of the control group. Data in each time-point represent replicates of 4 and are expressed as mean±SEM (P > 0.05).

**Intestinal transit time**

The possible effect of *R. damascena* on intestinal transit time was studied using cumulative concentration of phenol red within the intestine and geometric centre. The cumulative concentrations of phenol red in different parts of the intestine are shown in Figure 4. These represent the percentage of the gavaged contents passed each part of the small intestine at the given time. The results were not statistically different between the test and the control groups.

An alternative way to study the intestinal transit time is to calculate the geometric centre. Consistent to the results from cumulative concentration of phenol red, the results between the two experimental groups were not statistically different (Figure 5).

**Discussion**

In this research, the laxative/purgative effects of the boiled extract of *R. damascena* Mill. were investigated. An increase in feces water and the frequency of defeication are among the most important characteristics of laxatives (26). Oral gavaging of the extract at 1.5 g/kg Bwt significantly increased both parameters in rats, suggesting laxative effects for the plant.

Laxatives are among the most widely used drugs. However, their consumption is limited due to insufficient efficacy or the side effects, especially when used continuously or with contraindications. Bloating, cramping, diarrhea, and metabolic disturbances such as hypercalcemia, hyperphosphatemia, hyponatremia, and hypokalemia are among the most common side effects (22). Cardiototoxic and arrhythmogenic effects have been reported with magnesium purgatives (23) and cisapride (24). The use of stimulant laxatives such as senna compounds and bisacodyl may be associated with colonic neoplasia (25). The search for novel safe laxative drugs seems, therefore, inevitable.

A huge category of laxative agents, both with herbal and chemical origin, exert their effect via osmotic infiltration of fluids into the intestinal lumen. These drugs or their metabolites are slightly, if any, absorbed and increase osmolarity of intestinal contents (26). In this research, possible osmotic infiltration of fluids into the intestinal segments due to the boiled extract was studied in comparison to lactulose, a widely used osmotic laxative (26) and both significantly increased intestinal fluid contents compared to placebo (saline). This suggests the extract may induce its laxative effects, at least partly, via increased intestinal osmolarity. As an alternative justification, the extract may stimulate intestinal electrolyte secretion. In fact, one category of laxative drugs, known as stimulant or irritant laxatives, including bisacodyl, castor oil, senna, cascara etc, acts via increased electrolyte secretion from intestinal crypts (26). In order to rule out this possibility, a complementary experiment was performed, in which laxative effect of the extract was studied in orally gavaged rats in comparison to those received it intraperitoneally. To our amazement, instead of 22% increase in feces count in orally treated rats, the i.p. injected animals showed symptoms of constipation (no feces in 24 hr). It seems, therefore, the laxative effects of *R. damascena* extract relates, at least partly, to osmotic infiltration of fluids into intestinal lumen.

Laxative/purgative effects may be caused as a result of increased motility, and subsequently, decreased transit time in the intestine. This may arise as a result of enhanced stimulatory effects of neurohumoral substances, or suppressed inhibitory pathways, within the gut wall. Various neurohumoral effects such as antiepileptic...
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(12, 13), hypnotic (27, 28), antitussive (10), antinociceptive (15), bronchodilator (11) and hypoglycemic (14) properties have been described for *R. damascena*. These suggest that *R. damascena* extract may interfere with a wide variety of regulatory neurohumoral pathways, although the exact mechanisms are not yet discovered in most cases. On the other hand, varying regulatory substances are released from enteric nervous and endocrine systems that affect gastrointestinal movements and secretions (29, 30). It seems, therefore, conceivable that *R. damascena* extract affect gastrointestinal motility via affecting its regulatory neuronal or endocrine pathways. Consistently, our recent studies (unpublished data) suggest strong inhibitory effect on gastric emptying and intense stimulatory effect on gastric acid secretion for boiled extract of *R. damascena* in rats. Obviously, the above inhibitory effect on stomach and those reported on the bronchial smooth muscle cells (11) are in contrast to laxative properties of the plant observed in this research. It should be noted, however, that different mechanisms may be involved in intestinal smooth muscle cells and there is no report regarding the effects of *R. damascena* on these cells.

In this research, the boiled extract of *R. damascena* did not affect intestinal transit time. However, regarding its inhibitory effect on gastric emptying, its laxative effects may be, at least partly, due to reduction of intestinal transit time. In other words, the effects on intestinal transit time may be masked by delayed gastric emptying. This, however, demands further experiments.

This research, did not study the chemical ingredients involved in laxative effects of *R. damascena*. Actually *R. damascena* petals are mainly used in perfume industry and most studies on chemical ingredients of the plant are focused on rose essential oil. Chemicals such as geraniol, citronellol, farnesol, nerol, linalool present in rose oil are volatile (4), and therefore, do not seem to resist 10 min boiling in this research. Other ingredients, like querectin, multiflorine, kampferol (4), seem to be present in the extract and may account for some biological effects. Further research, however, is needed to verify these assumptions.

**Conclusion**

The current research showed significant laxative effects for boiled extract of *R. damascena* in rats. The effect seems to be, at least in part, due to osmotic infiltration of fluids into the intestinal lumen. Intestinal transit time of the marker, phenol red, did not change due to the extract. However, since the effect might have been masked by delayed gastric emptying, further research is required to assess possible prokinetic effects of the extract.

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**References**

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