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Original Article

Assessment of the Effect of the Combination of the *Pistacia vera* L. Gum Methanolic Extract and Ketamine on Anesthetic Parameters in Male Rats

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Abstract

Background and Aim: Several pharmacological properties, including muscle relaxant, and hypnotic as well as anxiolytic effects have been attributed to *Pistacia vera gum.* Hence, this study aimed to assess a blend of *Pistacia vera* gum methanolic extract and ketamine on anesthesia parameters in male rats.
Materials and Methods: In this study, 24 male rats (220-250 g) were randomly divided into 3 groups, including *Pistacia vera* extract alone (PV;500mg/kg, i.p.), *Pistacia vera*-ketamine (PVK;500mg/kg-80mg/kg, i.p., respectively), and diazepam-ketamine (DK;2.5 mg/kg-80 mg/kg, i.p., respectively) group (n=8). For this purpose, induction, and duration of surgical anesthesia (SA) walking time, heart and respiratory rate, body temperature, and withdrawal reflexes (pedal withdrawal, lip, and tail pinches) were evaluated during 55 min.
Results: The results of the present study showed that in both of the groups (except the PV group) SA was induced, and walking time in the PVK group was remarkably faster than the DK group ($p<0.05$). Heart rate was increased in the PVK group compared with the other two groups ($p<0.05$). Respiratory rates were decreased in the PV and PVK groups compared with the DK group ($p<0.05$). Body temperature in the PVK group decreased compared with the PV and DK groups ($p<0.05$). Lip and tail pinch were not significant between the PVK and DK groups, but in pedal pinch scores, the PVK group was better than the DK group in pain inhibition ($p<0.05$).
Conclusion: Anesthesia with PVK combination is preferred to a short time of anesthesia, and the pre-anesthetic properties of *Pistacia vera* gum extract are similar to diazepam. However, further studies are needed to identify its exact mechanism of action.
Keywords: *Pistacia vera gum*, Adjuvants, Anesthetic agents, GABAergic system

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Introduction

Sensation is eliminated during general anesthesia through controlled, reversible depression of the central nervous system (1). Human sensitivity and motor reactions to external noxious stimuli are decreased under general anesthesia (2). An optimal general anesthetic agent would induce these impacts

without the depression of the respiratory or cardiovascular systems, and provide desirable muscle relaxation. Moreover, it would be readily available, economical, non-irritant, stable, and non-toxic, and its clearance from the body would not depend on metabolism (1,3). Unfortunately, such an agent is not available. However, a balanced anesthetic technique can be used through the utilization of more than one drug to achieve the desired impacts of narcosis, muscle relaxation, and analgesia. Although the quality of anesthetic drugs used for laboratory animals has been noticeably enhanced in recent years, these drugs still cannot meet the criteria of ideal anesthetic agents. Thus, precise pre-anesthetic evaluation of patients prior to sedation and anesthesia is of high significance to determine physiological, pathological, or drug-related factors that might complicate the anesthetic management or the surgical procedure, the expected result of the surgery, or the management of the patient (1,3).

In humans, a combination of a sedative-hypnotic agent and ketamine, an anesthetic agent widely used in animal studies, is frequently used to enhance ketamine's anesthetic effects, reduce its side effects and also provide the necessary depth of anesthesia and surgical comfort (4). Unlike most of anesthetics, ketamine raises heart rate and mean arterial pressure, stimulates cardiovascular functions and when used alone it can cause optimal effects, including muscular hypertonicity, myoclonus, and convulsions (4). To reduce these unwanted and restricting impacts to the lowest levels, ketamine is given in combination with other agents, including benzodiazepines, and α_2 agonists. Diazepam is a potent hypnotic-sedative and induces muscle relaxation. Its slow metabolism makes it a long-acting drug, and it has relatively weaker cardiovascular impacts in comparison with other sedative agents (1). In this regard, our recent studies and other research demonstrate the anesthetic effects of different medicinal plants, including *Citrus aurantium L.*, *Humulus lupulus*, *Passiflora incarnate*, *Rosa damascene*, *Olea hochstetteri*, and *Myrtus communis* (5-10).

Pistacia vera L. (PV) belongs to the Anacardiaceae family and is native to the arid zones of Central and West Asia, and its fruits (pistachio) are used in traditional medicine (11). Previous studies have shown that PV has anti-nociceptive, anti-inflammatory (12), antioxidant (13), hepatoprotective (14), anticonvulsant (15), muscle relaxant, antianxiety and hypnotic activities (16). However, there is no evidence to confirm its effects on anesthesia parameters. Hence, this study aimed to investigate the effect of the combination of *Pistacia vera* and ketamine (PVK) on anesthesia parameters in male rats.

Materials and Methods

Preparation of the Methanolic Extract of *Pistacia vera* L

Pistacia vera gum was obtained from Rafsanjan (Kerman, Iran) and then (20 gm) was macerated in 200 ml of methanol. The plant material was soaked in methanol and kept for 48 hours. Subsequently, the extract was filtered and evaporated using a rotatory evaporator. The dried extracts were maintained under a nitrogen environment until further use. The percentage yield of the extract was 6.7% w/w dry matter.

Drugs

The drugs used included diazepam hydrochloride (Darupakhsh Pharmaceutical Co, Iran*)* and ketamine hydrochloride (Alfasan, Netherlands). The *Pistacia vera* gum extract was dissolved in normal saline (0.9%) with 1% Tween-80. In this study, all the drugs and *Pistacia vera* gum extract were administered intraperitoneally (i.p.) at a constant volume of 1ml/kg.

Animals

Twenty-four male Wistar rats weighing 220-250g were purchased from the Urmia University of Medical Science, and they were reserved in individual cages (one rat in each cage). The rats had free access to water, and were kept in a room at 23±2 $^{\circ}C$ with a fixed 12:12-light/dark period and a suitable humidity of 45-60%. In the present study, all the ethical principles were in accordance with the guidelines of the Urmia Branch, Islamic Azad University (No.10310501912074).

Experimental Design and Animal Grouping

After one week of accommodation, the rats were randomly divided into 3 groups (n=8 in each group) as follows:

1. *Pistacia vera gum* extract alone (PV): this group intraperitoneally(i.p.) received PV at a dose of 500mg/kg.
2. *Pistacia vera gum* extract and ketamine (PVK): this group i.p. received the combination of PV (500mg/kg)

and ketamine(80mg/kg).

3. Diazepam and ketamine (DK): this group i.p. received the combination of D (2.5 mg/kg) and ketamine (80mg/kg).

Anesthesia Parameters

In this study, induction time was recorded as the time needed for the loss of righting reflex. Duration of surgical anesthesia (SA) was defined and recorded as the time needed for the loss of pedal withdrawal reflex. Moreover, walking time was considered as the time needed for the loss of righting reflex until the ability to walk. The rats were laid in dorsal recumbency after the loss of righting reflex.

The respiratory rate was calculated via the observation of the thoracic movements and heart rate was computed through ECG recording (*Bionet, Japan*): lead II, by hypodermic needle electrode, paper speed 50 mm/sec, for the 5 intervals during 60 min after administration until the righting reflex returned. Rectal temperature was measured by a digital thermometer inserted at least 3 cm into the rectum at 5 minutes' post-administration intervals. Body temperature was maintained at (37-38)°C during anesthesia using a heat lamp.

The depth of anesthesia was assessed by withdrawal reflexes (pedal withdrawal, and lip as well as tail pinch reflexes) every five minutes as described by Hedenqvist *et al*. (17). The withdrawal reflexes were evaluated by pinching the interdigital of the hind foot. The lower lip reflexes were measured by traumatic plastic forceps, and the distal part of the tail was assessed with the thumb and index finger. Responses were scored on a four-point scale (0-3), with completely absent reflexes scored as 0, relatively weak reflexes as 1, medium reflexes as 2, and strong withdrawal responses as 3. All the protocols have already been described by Hedenqvist *et al*. (17).

Statistical Analysis

In the present study, the data were expressed as mean±standard deviation. The quantitative data were analyzed using one-way variance analysis and the Tukey's post hoc test, and the qualitative data were analyzed using the Kruskal-Wallis test. The data were analyzed in SPSS-20 and graphs were plotted in Excel-2016 at a significance level of $p<0.05$.

Results and Discussion

As it has been shown in Table 1, the surgical anesthesia

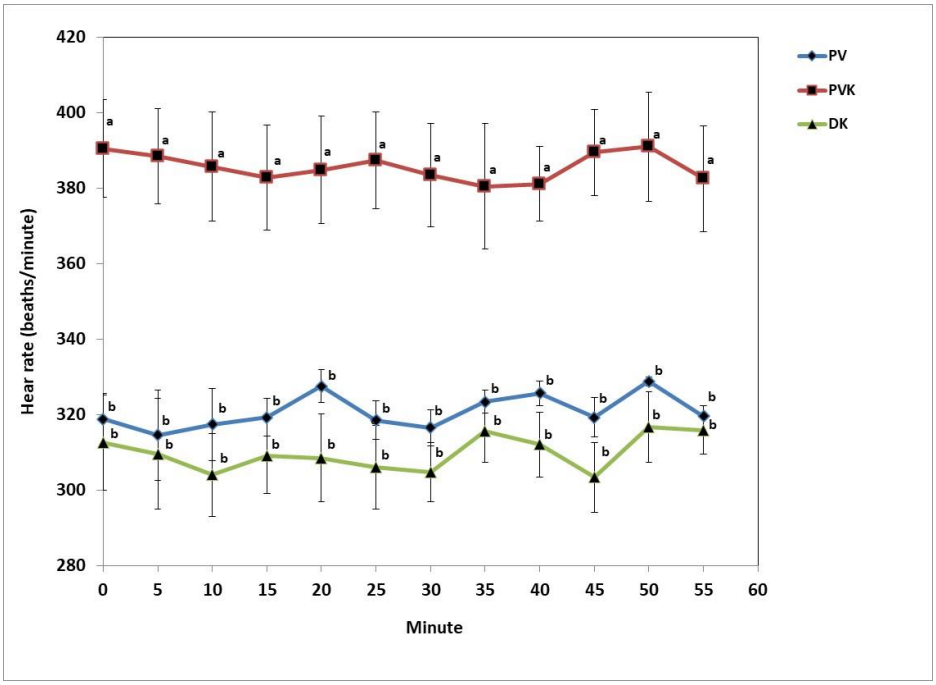

Figure 1: Effects of *Pistacia vera* (PV), *Pistacia vera* plus Ketamine (PVK), and Dizepam plus ketamine (DK) groups on heart rate. Values are given as mean ± SEM (*n*=8). Different letters are indicative of a significant difference between the groups at $p<0.05$.

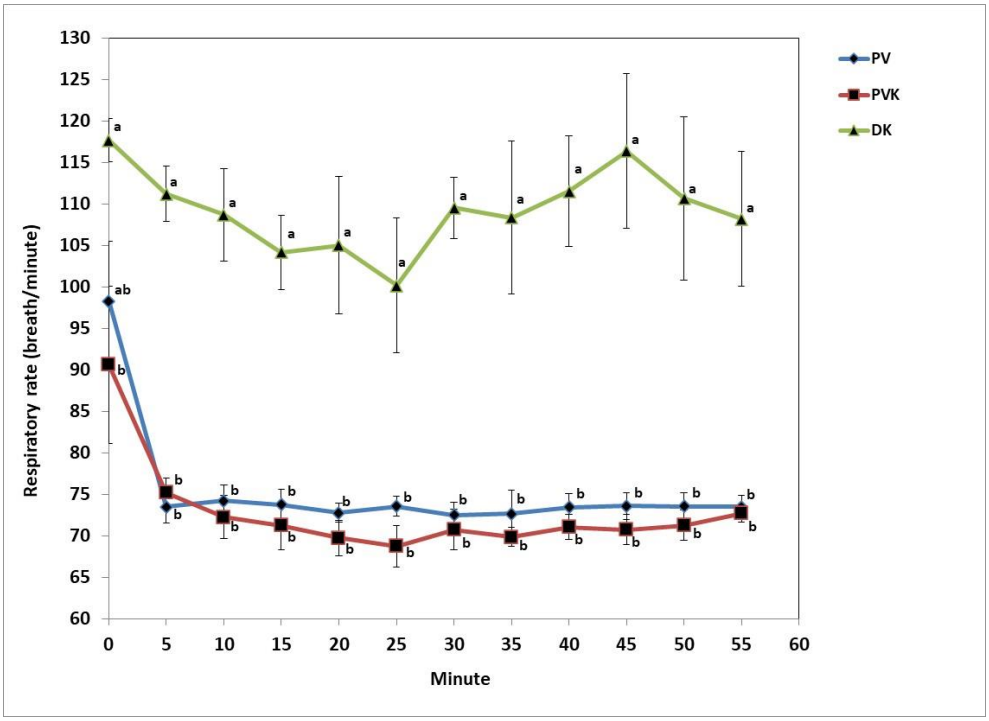

Figure 2: Effects of *Pistacia vera* (PV), *Pistacia vera* plus Ketamine (PVK), and Dizepam plus ketamine (DK) on respiratory rate. Values are given as mean ± SEM (*n*=8). Different letters are indicative of a remarkable distinction between the groups at p<0.05.

Table 1: Effects of *Pistacia vera* (PV), *Pistacia vera* plus Ketamine (PVK), and Dizepam plus ketamine (DK) groups on induction time, duration of surgical anesthesia and, walking time.

Reflex Group	Induction Time	Duration of Surgical Anesthesia	Walking Time
DK	5±0.7 a	52.5±4.76 a	77.25±8.59 a
PV	-	-	-
PVK	7±1.08 a	31.75±1.37 a	38.75±1.37 b

Values are given as mean ± SEM (*n*=8). Different letters indicate a significant difference between the groups at p<0.05.

(SA) was not induced in the PV group. However, the DK group exhibited an insignificantly faster induction time span and a long duration of SA than the PVK group (p>0.05). Moreover, the DK group exhibited significantly a late waking time compared with the PVK group (p<0.05). Consistent with our results, the results of the study conducted by Ziaee and Hoseinzadeh confirmed the hypnotic and muscle relaxant effects of the PV extract in mice. They showed that all doses (0.25-1 g/kg) of the PV extract similar to diazepam (1mg/kg) decreased the onset of sleeping and increased the duration of sleep. Furthermore, they indicated that only the high dose (1g/kg) of the extract could significantly decrease sleep latency (16). Despite this, Parvardeh *et al.* (2002) showed that the lethal dose 50 (LD_{50}) of *Pistacia vera* gum hydroalcoholic extract was 3.77g/kg in rats (15).

As it has been illustrated in Figure 1, the PV and PVK groups had the lowest heart rates compared with the DK group (p<0.05). Moreover, there was no significant difference between the heart rates of the PV and PVK groups (p>0.05). On the other hand, when ketamine was used alone it could increase heart rate and mean arterial pressure and stimulate cardiovascular functions (3). Previous studies have demonstrated the cardioprotective effects of PV. Furthermore, the PV phytochemical antioxidants (including monounsaturated fatty acids and fibers) could promote heart health (18).

As it has been shown in Figure 2, the PV and PVK groups had the lowest respiratory rate compared with the DK group (p<0.05). Moreover, there was no significant difference between the respiratory rate of the PV and PVK groups (p>0.05). Previous studies have shown that the Anacardiaceae family (including PV) has beneficial effects in the treatment of respiratory

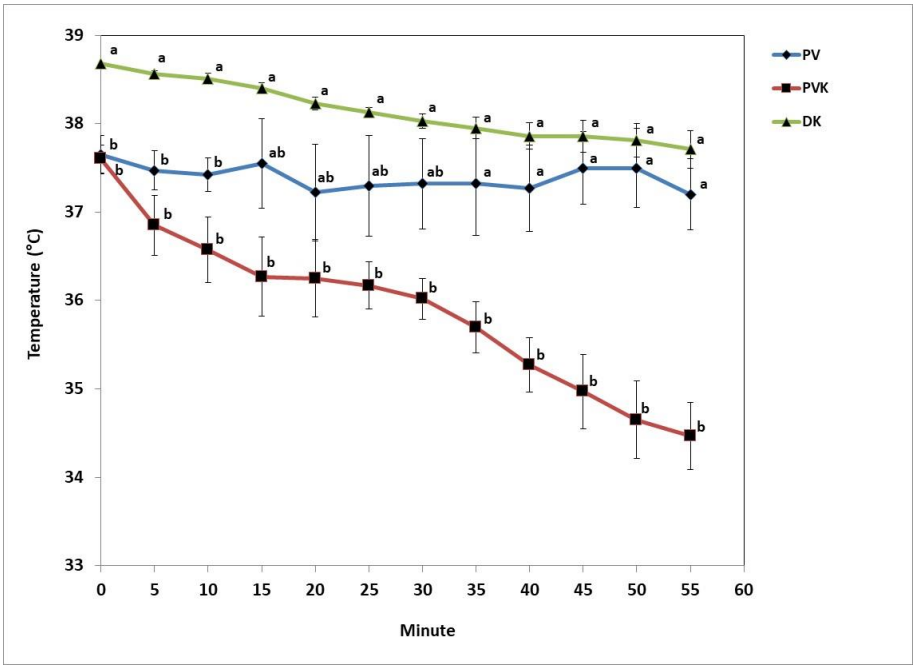

Figure 3: Effects of *Pistacia vera* (PV), *Pistacia vera* plus Ketamine (PVK), and Dizepam plus ketamine (DK) on body temperature. Values are given as mean ± SEM (*n*=8). Different letters mean a significant distinction between the groups at p<0.05.

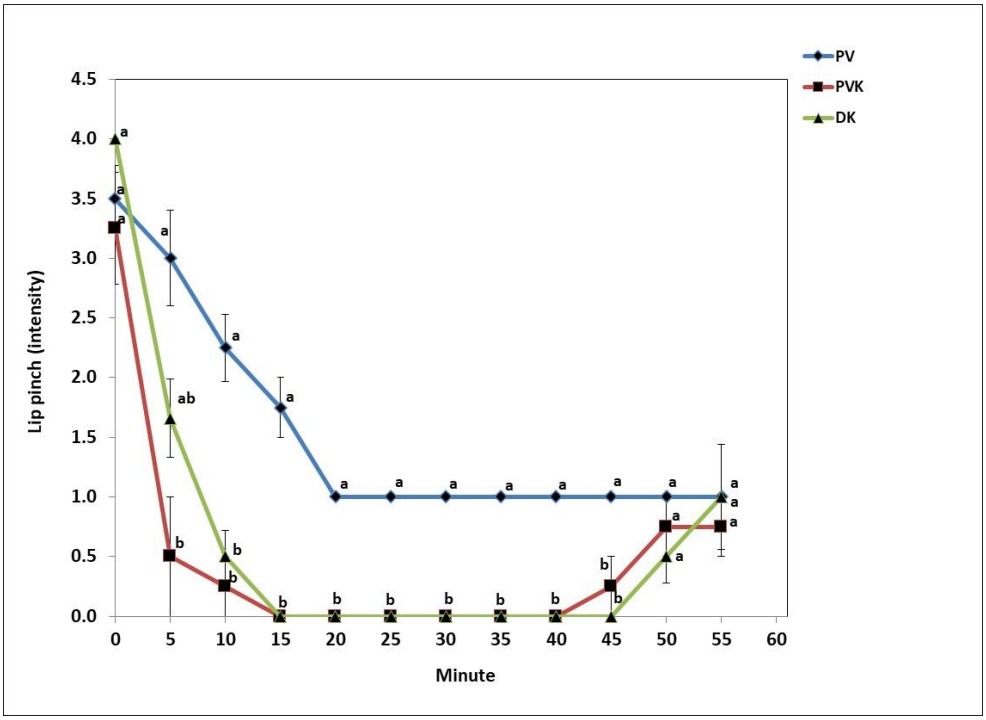

Figure 4: Effects of *Pistacia vera* (PV), *Pistacia vera* plus Ketamine (PVK), and Dizepam plus ketamine (DK) groups on lip pinch. Values are given as mean ± SEM (*n*=8). Different letters are indicative of a significant difference between the groups at p<0.05.

diseases (19).

As it has been illustrated in Figure 3, the PV and PVK groups have the lowest temperature compared with the DK group (p<0.05). Moreover, there was no significant

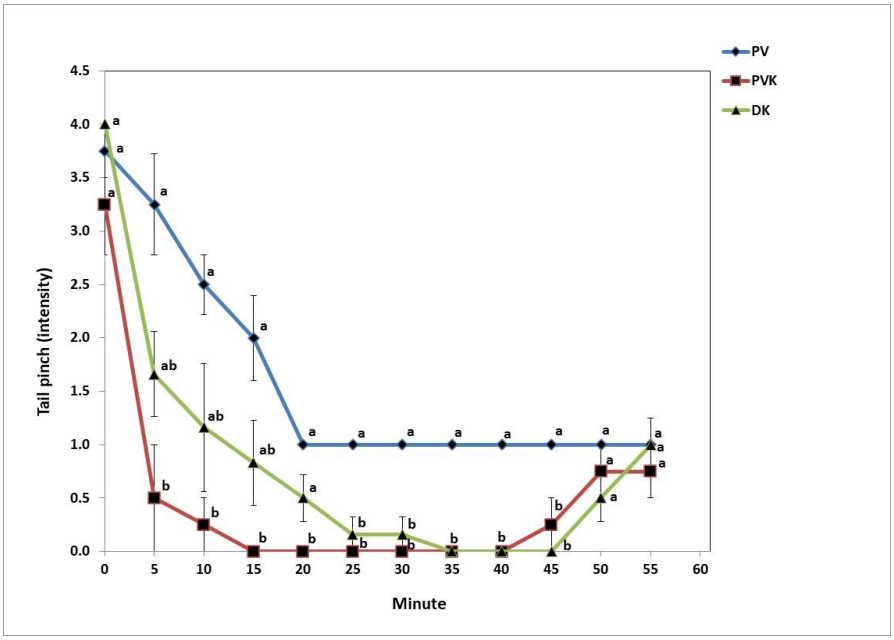

Figure 5: Effects of *Pistacia vera* (PV), *Pistacia vera* plus Ketamine (PVK), and Dizepam plus ketamine (DK) on the tail pinch. Values are given as mean ± SEM (*n*=8). Different letters indicate a significant difference between the groups at p<0.05.

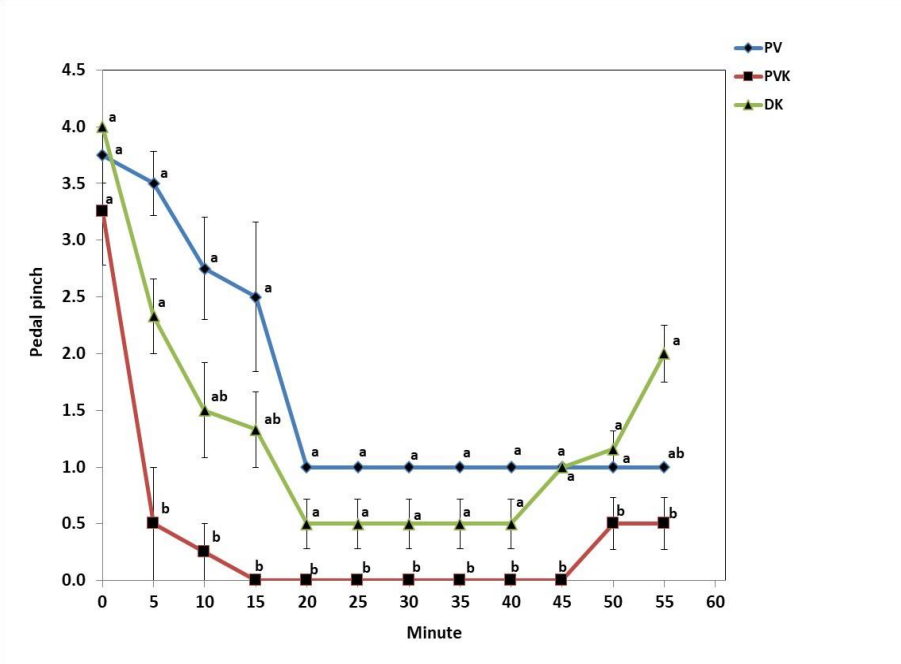

Figure 6: Effects of *Pistacia vera* (PV), *Pistacia vera* plus Ketamine (PVK), and Dizepam plus ketamine (DK) on the pedal pinch. Values are given as mean ± SEM (*n*=8). Different letters show a significant difference between the groups at p<0.05.

difference between the temperature of the PV and PVK groups (p>0.05). Consistent with our results, previous studies indicated the antipyretic properties of the Anacardiaceae family (19).

As it has been illustrated in Figure 4, the PV and PVK groups inhibited the lip pinch significantly better than the DK group (P<0.05). However, there was no significant difference between the PV and PVK groups (p>0.05).

As it has been shown in Figure 5, the PV and PVK groups inhibited the tail pinch significantly better than the DK group (p<0.05). However, there was no

remarkable difference between the PV and PVK groups ($p>0.05$).

As it has been illustrated in Figure 6, the PVK group inhibited the pedal pinch significantly better than the DK group ($p<0.05$). However, there was no significant difference between the PV and PVK groups ($p>0.05$). Consistent with our results, different studies have indicated the antinociceptive property of PV (20,21). Moreover, Hosseinzadeh *et al*. (2011) reported that the i.p. administration of the aqueous and ethanolic extracts of the *PV* leaves (at the doses of 400 and 500mg/kg, respectively) showed dose-dependently central and peripheral antinociceptive activities (20). In another study, Parvardeh *et al.* (2002) indicated the antinociceptive properties of the ethanolic extract of the PV gum (at doses of 250,500 and 1000mg/kg) (21).

Conclusion

In summary, anesthesia with PVK combination is preferable to short-time anesthesia, and the pre-anesthetic properties of *Pistacia vera* are similar to diazepam. Further studies are needed to determine its exact mechanism of action.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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