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ANTI-NOCICEPTIVE EFFECT OF METHANOL EXTRACT OF ROOTS OF *SARCOPOTERIUM SPINOSUM* IN MICE

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Abstract

Sarcopoterium spinosum.L (Rosaceae) is a medicinal plant traditionally used in Jordan folk medicine for the treatment of diabetes, toothaches, digestive problems, inflammation, and pain. This study was conducted to establish the antinociceptive activity of methanol extract of roots of *Sarcopoterium spinosum* using heat-induced (hot-plate) and chemical-induced (acetic acid and formalin) nociception models in mice.

The *Sarcopoterium spinosum* methanolic extract at test doses of 150 and 300 mg/kg, i.p. clearly demonstrated antinociceptive activity in all tests. In all models, the combination of *Sarcopoterium spinosum* with either diclofenac or morphine produced statistically significant increase ($p \leq 0.05$) in the percentage of inhibition of writhing, paw licking, and %MPE compared to single treatment groups. It was also found that the 300 mg/kg extract produced higher antinociceptive effects ($p \leq 0.05$) compared to the 150 mg/kg.

Sarcopoterium spinosum roots may have analgesic effect that supports previous claims of its traditional use. The analgesic effect is mediated through both peripheral and central mechanisms.

Keywords: Antinociceptive, *Sarcopoterium spinosum*, hotplate test, formalin test.

Introduction

Jordan has tremendous wealth of medicinal plants scattered in a vast area. These plants are used in Jordan folklore medicine for their medical as well as nutritive values.

Sarcopoterium spinosum L. is one of the thorn plants, it is widely grown in the Mediterranean region, belong to Rosaceae family and its roots are widely used as an antidiabetic drug by Bedouin healers [1]. In Jordan *Sarcopoterium spinosum* spreads naturally in South regions and their roots are used for the treatment of diabetes, toothaches, digestive problems, inflammation, and pain [2]. Phytochemically, the aerial and underground parts of *Sarcopoterium spinosum* were reported to contain triterpenoids such as ursolic acid, tormentic acid, and sitosterol while the leaves were reported to have carotenoids, flavonoids, and its derivatives such as catechin, epicatechin, quercitrin, quercetin, and hyperoside [3-5].

Considering the popular use and because of the scarcity of pharmacological studies supporting analgesic potential of *Sarcopoterium spinosum* the present study aimed to investigate the antinociceptive effect of methanol extract of roots of *Sarcopoterium spinosum* in mice

Methods

Morphine Sulfate (MEDOCHEMIE, Cyprus) was used as a positive control during the study (hot-plate test and formalin-induced paw licking test). It was administered at dose of 5 mg/kg. *Sarcopoterium spinosum* extract was administered at doses of 150 mg/kg and 300mg/kg. Diclofenac Sodium (MEDOCHEMIE, Cyprus) was used as a positive control for acetic acid-induced writhing test. All other chemicals and drugs used in this study were of analytical grade

Experimental animals

Non-fasted male Swiss albino mice (24-28 g) housed at 22-25° under a 12 h light/dark cycle and with access to food and water *ad labium*, were used throughout the experiments. Efforts were made to minimize the number of animals used and their sufferings. The experiments were carried out in accordance with the current ethical and care guidelines for the care of laboratory animals and the investigation of experimental pain in conscious animals. The experiments were approved by the local Research Ethics Committee of the Hashemite University. On the day of testing, mice were removed from the animal care facility to testing area 1 h before testing and placed separately to decrease stress and decrease variability between mice, animals were handled for 5 min before experiments. All drugs and test solutions were administered in a volume of 10 ml/kg.

Plant material

The roots of *Sarcopoterium spinosum* were collected during May 2018 from Al-Tafila (Jordan). The plant material was identified and authenticated at the Faculty of Agriculture, The University of Jordan. A voucher specimen was deposited at the Hashemite University herbarium, Zarqa, Jordan, for future reference. The roots of the plant were sorted, cleaned, & dried at room temperature. Powdered plant material (500 g) was soaked in 2000 ml of petroleum ether (60-80°) for 10 days at room temperature for defatting. The plant material was separated by filtration using cheesecloth. The marc was then allowed to dry and extracted three times by maceration using 80% methanol with intermittent agitation, each maceration step being carried out for 3 days. The extracts were filtered, collected, concentrated under reduced pressure using a rotary evaporator (Heidolph Model Laborota 4000, Germany) and dried in a vacuum oven at 35°C. The dried extracts were then transferred into vials and stored for further use.

Acute oral toxicity testing

Mice were used to determine the intraperitoneal LD_{50} values of methanol extract of *Sarcopoterium spinosum*. The study animals (n = 20) were divided into four groups of five mice per cage. Before the administration of a single dose of the extract, the mice fasted for 2 hours. First, an acute oral toxicity study was performed on five mice (weight, 30-32 g). The mice were given 2000 mg/kg (i.p) of the extract dissolved in distilled water. The test was then performed on the remaining 15 mice (male; weight, 23-25 g), which were divided into three groups of five each: The first group (5 mice) was given 0.5 ml of distilled water, whereas the second (5 mice) and third (5 mice) groups received the root extract of *Sarcopoterium spinosum* (5000 mg/kg dissolved in distilled water). Then, the

animals were continuously observed during the first 30 minutes, periodically observed during the first 24 hours, with particular attention during the first 4 hours, and observed daily thereafter. The animals were observed for 14 days for gross behavioral changes such as loss of appetite, hair erection, lacrimation, tremors, convulsions, salivation, diarrhea, mortality, and other signs of toxicity manifestation [6].

Acetic acid-induced writhing

This test was conducted as per the method described by Koster et al. (1959)[7]. 42 Swiss mice were randomly divided into six groups of, seven mice per group. Group I were treated with 0.5ml of normal saline and served as control; group II with diclofenac sodium (10 mg/kg); groups III & IV received methanol extract of *Sarcopoterium spinosum* (150 or 300 mg/kg respectively), and groups V & VI receive combinations of *Sarcopoterium spinosum* extract (150 or 300 mg/kg) plus diclofenac sodium (10 mg/kg). All treatments were administered i.p.. Thirty minutes after treatment, each mouse was administered with 10 ml/kg of 0.6% acetic acid (i.p.) and the number of writhing displayed by each mouse was counted and recorded for 30 minutes. The contractions of the abdomen, elongation of the body, twisting of the trunk and/or pelvis ending with the extension of the limbs were considered as complete writhing. Results were expressed as mean percent inhibition of writhing (PIW):

PIW = (# writhing of control - # writhing of treatment/ # writhing of control) X 100

Hot-plate test

The test was performed using a hot-plate analgesiometer maintained at 55± 0.5◦C. 42 mice that showed nociceptive responses within 10 seconds when placed on the hot plate were selected and randomly divided into six groups, seven mice per group. Group I were treated with 0.1ml of normal saline and served as control; group II with morphine sulfate (5 mg/kg); groups III & IV received methanol extract of *Sarcopoterium spinosum* (150 or 300 mg/kg, respectively), and groups V & VI receive combinations of *Sarcopoterium spinosum* extract (150 or 300 mg/kg, respectively) plus morphine (5 mg/kg). All treatments were administered i.p. Each mouse was gently placed on the plate and latency of mice to the thermal stimulus was recorded at 20, 30, 45, 60, 90 and 120 minutes post-treatment. Reaction time was taken as the interval between the instant the animal was placed on the plate till the moment it began to lick its paws. fourty seconds was chosen as the cut off time to avoid tissue damage. Results were expressed as mean percent maximal effect (%MPE) [8]:

%MPE = (Post drug latency – Pre drug latency/ Cut-off time – Pre drug latency) X 100

Formalin-induced paw licking test

The test was carried out as per the method described by Tjolsen et al. (1992) [9]. 42 Swiss mice were randomly divided in to six groups, seven mice per group. Group I were treated with 0.1ml of normal saline and served as control; group II with morphine sulfate (5mg/kg); groups III & IV received methanolic extract of roots of *Sarcopoterium spinosum* (150 or 300 mg/kg, respectively), and groups V & VI receive combinations of *Sarcopoterium spinosum* extract (150 or 300 mg/kg, respectively) plus morphine (5 mg/kg). All treatments were administered (i.p) 60 minutes before injecting formalin. Pain was induced by injecting 20 µl of 2.5% formalin into the sub-plantar region of the right hind paw. The amount of time spent licking the injected paw was calculated and considered as an indication of pain. Licking of the injected paw was recorded as nociceptive response at 0-5 min and 15-30 min after formalin injection representing the two phases of pain response; neurogenic and inflammatory phase, respectively. Results were expressed as mean percent inhibition of licking response (PIL):

PIL = (Time spent licking for control – Time spent licking for treatment/ Time spent licking for control) X 100

Statistical analysis

Values are expressed as the mean±SE and all data were analyzed using ANOVA followed by Bonferroni test for multiple comparisons for means.

Results

Acetic acid-induced writhing test

In the present study, the extract significantly (p≤0.05) decreased the frequency of acetic acid induced abdominal constriction (writhing) in mice and increased the percentage of inhibition of writhing response at both dosages administered (Table 1 and Figure 1). The combination of diclofenac with *Sarcopoterium spinosum* (300 mg/kg) produced significant increase (p≤0.05) in the percentage of inhibition compared to single treatment groups with diclofenac or the extract (Table 1 and Figure 1).

Hot-plate test

Sarcopoterium spinosum extract, at both dosage levels, also significantly (p≤0.05) increased the percentage of mean maximal effect (%MPE) compared to control group in the hot-plate test. The combination of the extract with morphine (5 mg/kg) was found to be superior to all single treatment groups at all time intervals of response measurement (p≤0.05) (Figure 2). It was also found that the 300 mg/kg extract produced higher effects (p≤0.05) compared to the 150 mg/kg.

Formalin-induced paw licking test

In the current study, *Sarcopoterium spinosum* produced anti-nociception in both phases of the formalin test (Figure 3). And similar to the above tests the combination of extract with morphine resulted in statistically significant increase (p≤0.05) in the percent inhibition of licking response in both phases of nociception (Figure 3). It is also noted that combination of morphine and *Sarcopoterium spinosum*
(300 mg/kg) extract was superior in its analgesic effect compared to all single treatments with morphine or extract.

Discussion

In the present study, the analgesic effect of the methanol extract of roots of *Sarcopoterium spinosum* using different pain models was assessed in mice and it was found that the extract has significant analgesic activity at both doses: 150 mg/kg, 300 mg/kg. Besides, the study revealed that the antinociceptive effect observed by the combination of the extract with standard drugs was superior to the respective single treatment groups. We believe that we are the first, to the best of our knowledge, to study the anti-nociceptive effect of the extract alone and in combination with standard treatments.

Acetic acid-induced abdominal constriction test is used to assess the peripheral antinociceptive activities of natural products [10] In the current study, the methanol extract of the roots of *Sarcopoterium spinosum* significantly increased the percentage of inhibition of writhing response compared to the control group. Besides, the combination of diclofenac with *Sarcopoterium spinosum* extract (300 mg/kg) produced significant increase (p≤0.05) in the percentage of inhibition compared to all single treatment groups (Figure 1). The different mediators of abdominal writhing in this model include acid sensing ion channels [11], peritoneal mast cells [12], and prostaglandin pathways [13]. Hence, the antinociceptive action of *Sarcopoterium spinosum* extract in this method may be due to inhibitory action against the synthesis and release of the mediators produced by these cells.

The hot-plate is a useful model for evaluation of centrally acting analgesics, such as opioid analgesics, which are known to raise the pain threshold of mice towards heat [14]. In the present study, *Sarcopoterium spinosum* significantly increased the percentage of mean maximal effect (%MPE) compared to control group in the hot-plate test at both doses administered. Further, the combination of the extract with morphine (5 mg/kg) was found to be superior to all single treatment groups at all intervals of response measurement (p≤0.05). It was also found that the 300 mg/kg extract produced highest effects compared to the 150 mg/kg (Figure 2). Hence, the ability of the extract to significantly increase the %MPE suggests that the extract possesses strong analgesic activity that could be mediated centrally.

The nociceptive behavior after formalin injection was recorded in two phases: neurogenic pain (first phase) and inflammatory pain (second phase). Our study showed that *Sarcopoterium spinosum* produced antinociceptive effect at both phases of the formalin test. And similar to the above tests the combination of extract with morphine resulted in statistically significant increase (p≤0.05) in the percent inhibition of licking response in both phases of nociception compared to all single treatments with morphine or extract (Figure 3). It is also noted that combination of morphine and *Sarcopoterium spinosum* (300 mg/kg) extract was superior to all other treatment groups in its analgesic effect.

Studies revealed that centrally acting analgesics inhibit equally both phases, while peripherally acting drugs suppress mainly the late phase [15-16].

Thus, *Sarcopoterium spinosum* roots may have analgesic effect that is mediated through both peripheral and central mechanisms and could be used as adjuvant treatment to the modern analgesics

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Table 1. Effect of Methanolic extract of *Sarcopoterium spinosum* (150 mg/kg or 300 mg/kg) alone and in combination with diclofenac sodium (10 mg/kg) on the number of writhing response on acetic acid-induced writhing test in mice.

Group #	N	# Writhing Response
Control	7	35.3 ± 1.6
Diclofenac	7	4.6 ± 0.58 •
S. spinosum 150mg/kg	7	10.1 ± 0.95 •
S. spinosum 300mg/kg	7	6.3 ± 0.94 •
Diclofenac + *S. spinosum* 150mg/kg	7	4.1 ± 0.62 •, #
Diclofenac + *S. spinosum* 300mg/kg	7	1.3 ± 0.44 •, *, #

• Significantly different form control at p≤0.05; ***** Significantly different from diclofenac at p≤0.05;
Significantly different from *S. spinosum*, 150 mg/kg or 300 mg/kg at p≤0.05.

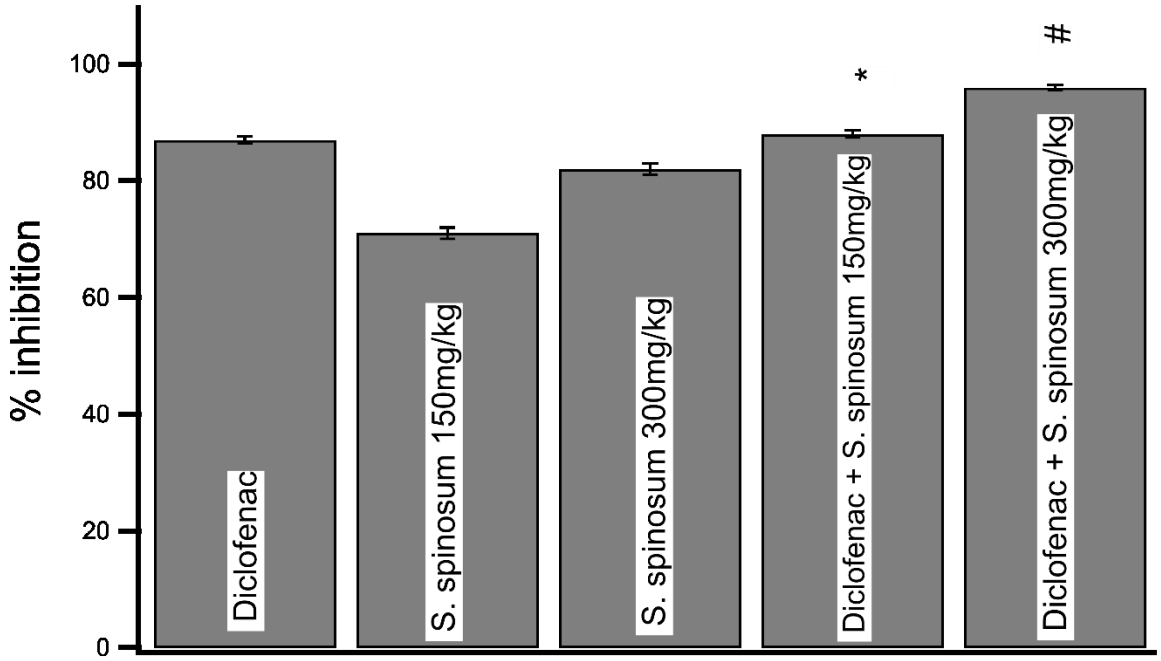

Figure 1. Effect of Methanolic extract of *S. spinosum* (150 mg/kg or 300 mg/kg) alone and in combination with diclofenac sodium (10 mg/kg) on percent inhibition of writhing response on acetic acid-induced writhing test in mice. Number of animals per group (n) =7. ***** Significantly different from Diclofenac at p≤0.05. # Significantly different from *S. spinosum*, 150 mg/kg or 300 mg/kg at p≤0.05.

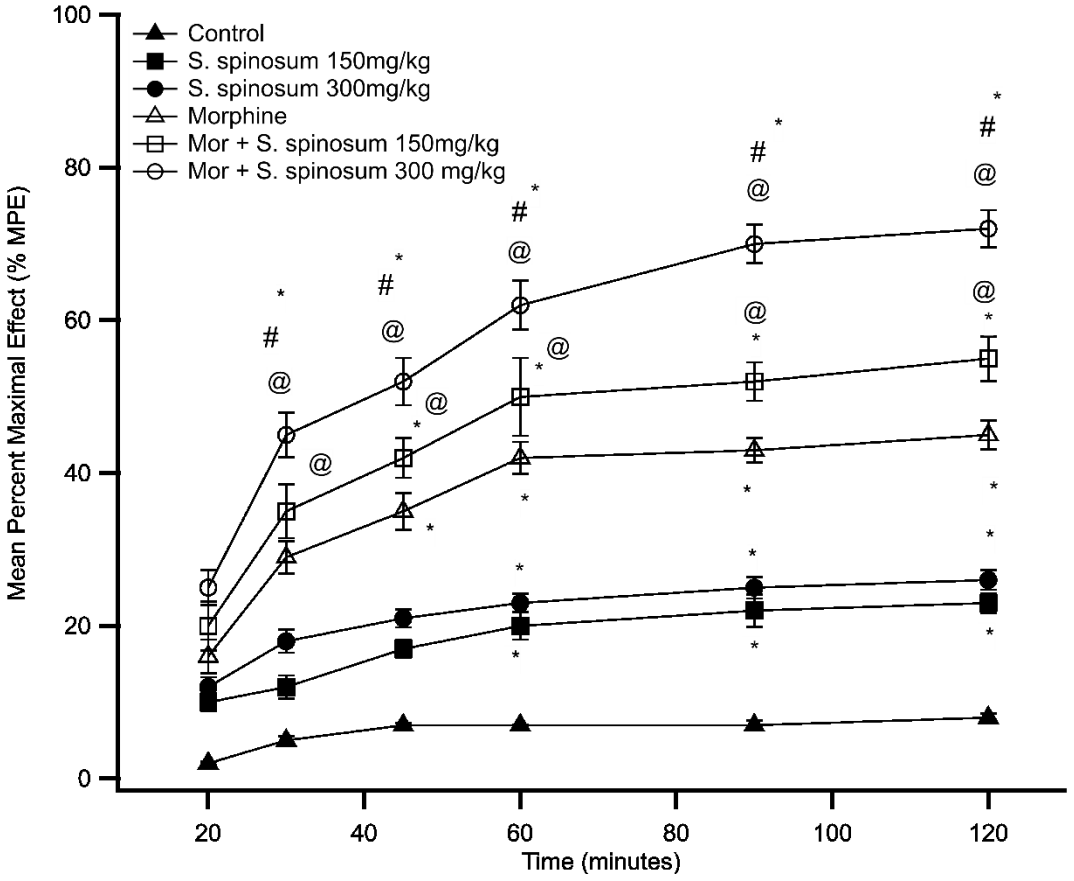

Figure 2. Effect of methanolic extract of *S. spinosum* (150 mg/kg or 300 mg/kg) alone and in combination with morphine (5 mg/kg) on mean percent maximal effect (%MPE) on hot plate test in mice. Number of animals per group (n) =7. ***** Significantly different form control at p≤0.05. # Significantly different from morphine at p≤0.05. @ Significantly different from *S. spinosum*, 150 mg/kg or 300 mg/kg at p≤0.05.

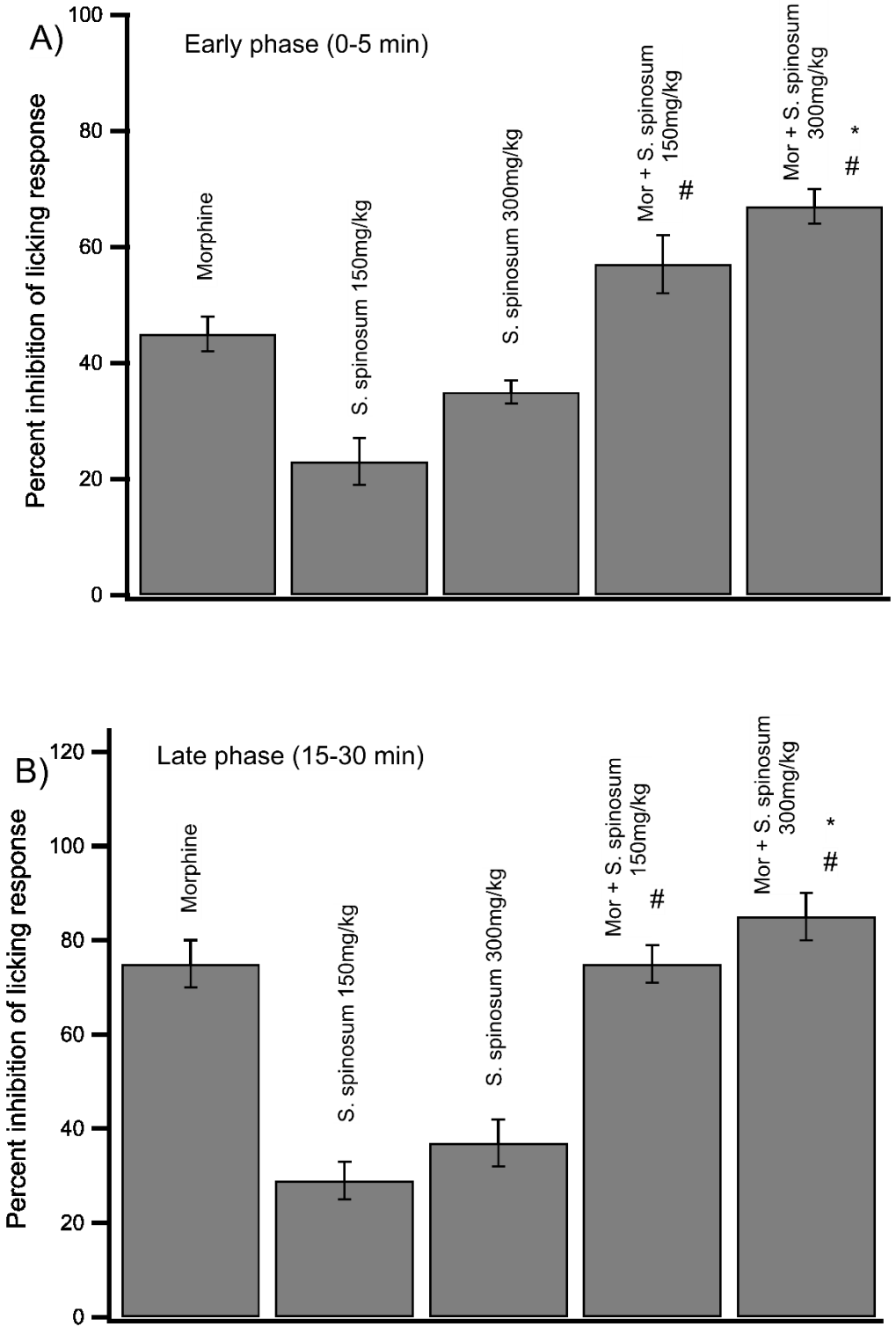

Figure 3. Effect of methanolic extract of *S. spinosum* (150 mg/kg or 300 mg/kg) alone and in combination with morphine (5mg/kg) on percent inhibition of licking response on formalin-induced paw licking test in mice.. Number of animals per group (n) =7. * Significantly different from morphine at p≤0.05. # Significantly different from *S. spinosum*, 150 mg/kg or 300 mg/kg at p≤0.05.