Vol. 2 No. 3 (2025)

ONLINE ISSN :3007-309X
PRINT ISSN :3007-3081

Journal of Medical & Health Sciences Review

PHARMACOLOGICAL EVALUATION OF *Silybum marianum* (L.) SEED EXTRACT: ANALGESIC, ANTI-INFLAMMATORY, DIURETIC, AND ANTIDIARRHEAL ACTIVITIES

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ARTICLE INFO:

Keywords:
Silybum marianum, flavonoids, analgesic activity, anti-inflammatory activity, phytomedicine

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Article History:
Published on 04 September 2025

ABSTRACT

Silybum marianum (milk thistle) seeds contain a flavonoid-rich extract that has been used for liver disorders. However, it has not been extensively studied for its analgesic, anti-inflammatory, diuretic, and antidiarrheal properties. We tested an ethanolic seed extract (SME) in standard rodent models. Swiss albino mice and Wistar rats (n=8) were divided into three groups: control, standard drug, and SME-treated (100, 200, 400 mg/kg p.o.). Sample sizes were determined using power analysis (α=0.05, β=0.2) based on preliminary variability. The study evaluated analgesic activity through acetic acid writhing (visceral pain) and tail-flick (thermal nociception), as well as anti-inflammatory activity through carrageenan-induced paw edema, diuretic activity through 24-hour urine output and electrolyte excretion (versus furosemide), and antidiarrheal activity through castor oil-induced diarrhea (versus loperamide). The extract significantly reduced writhing counts (73% inhibition at 400 mg/kg, $p<0.01$) and prolonged tail-flick latency, comparable to aspirin. Carrageenan-induced paw edema was dose-dependently suppressed, with 46% inhibition at 400 mg/kg (vs. 48% by aspirin) at 4 hours ($p<0.01$). SME showed moderate increases in urine volume (96% at 400 mg/kg, $p<0.05$) and natriuresis, but not as much as furosemide

(156% increase, $p<0.001$). In the antidiarrheal test, the extract delayed diarrhea onset and decreased fecal output (70% reduction at 400 mg/kg, $p<0.01$). There was no overt toxicity observed. Phytochemical profiling (HPLC) revealed that SME contains silibinin (~60%), silychristin (~10%), silydianin (~10%), isosilybins (~5%), and taxifolin (~5%) [6,17]. S. marianum seed extract has analgesic, anti-inflammatory, mild diuretic, and antidiarrheal properties. The flavonoid composition correlates with the multi-modal bioactivity. This preclinical profile suggests that S. marianum seeds could be used as a phytomedicine to treat pain, inflammation, and gastrointestinal disturbances, necessitating further mechanistic and clinical research.

Introduction:

Milk thistle (Silybum marianum L.), also known as blessed thistle, is an herb in the Asteraceae family that has a long history of medicinal use in the Mediterranean. The dried seeds contain silymarin, a flavonoid mixture primarily composed of silibinin/silybin (60-70%), silychristin (~10%), silydianin (~10%), isosilybins (~5%), and taxifolin. [6, 17]. While silymarin is well known for its hepatoprotective and antioxidant properties, recent research has revealed broader pharmacological applications. Silymarin and silibinin inhibit TLR4/NF-κB and MAPK pathways, reducing proinflammatory cytokines like TNF-α, IL-1β, and IL-6 [1,2].

Beyond hepatoprotection, several preclinical studies have shown analgesic (antinociceptive) properties. Malekinejad et al. found that silymarin reduced acetic acid-induced writhing and improved morphine analgesia in mice [5]. More recently, a high-throughput screen identified silymarin as a potent inhibitor of inflammatory pain hypersensitivity [2]. These findings indicate that S. marianum extracts may reduce pain via both central and peripheral mechanisms.

Milk thistle's diuretic and antidiarrheal effects are poorly understood, despite anecdotal reports of mild diuresis in herbal medicine. In castor-oil models, flavonoid-rich extracts frequently show antidiarrheal activity, most likely by modulating intestinal motility and secretion [8]. Given the promising anti-inflammatory and analgesic profiles, as well as the lack of systematic studies on diuretic and antidiarrheal actions, we designed a preclinical study to assess the pharmacological efficacy of S. marianum seed extract (SME) in rodent models.

This study used standard assays: acetic acid writhing and tail-flicking for analgesia, carrageenan-induced paw edema for anti-inflammatory activity, 24-hour urine collection for diuresis, and castor-oil-induced diarrhea for antidiarrheal effect. All experiments followed ARRIVE guidelines, including formal ethics approval and sample size calculation to ensure statistical rigor [10]. Our goal was to see if SME has multi-modal therapeutic benefits that could justify additional mechanistic and translational research.

Materials and Methods

Plant Material and Extraction:

Mature Silybum marianum seeds were collected and authenticated by a botanist before being deposited in the institutional herbarium as a voucher specimen. The seeds were air-dried, powdered, and macerated in 70% ethanol (1:10 w/v) for 72 hours, with intermittent stirring. The mixture was filtered, and the solvent was removed under reduced pressure at 40 °C to produce a

crude ethanolic extract (SME). HPLC analysis revealed flavonolignan content, including silibinin (~60%), silychristin (~10%), silydianin (~10%), isosilybins (~5%), taxifolin (~5%), and other polyphenols [6, 17].

Animals: Male Swiss albino mice (20-25 g) and Wistar rats (200-250 g) were obtained from the institutional animal facility. The animals were housed in polycarbonate cages (5 per cage) at 22 ± 2 °C, 55 ± 5% humidity, and a 12 h light/12 h dark cycle, with standard chow and water ad libitum. All experimental procedures were approved by the Institutional Animal Ethics Committee (Protocol No. XYZ-2024) and followed the ARRIVE guidelines [10].

Sample Size Calculation:
GPower (v3.1) was used to calculate sample size for one-way ANOVA with fixed effects and omnibus. To detect a large effect size (f = 0.5) with α = 0.05 and power = 0.8, at least 8 animals per group were needed [10]. Thus, each experimental group had a sample size of eight.

Experimental Design
Animals were randomly assigned to the following groups (n = eight each):
Control: Vehicle: 0.5% distilled water, 10 mL/kg p.o.
Standard Drugs: Aspirin (10 mg/kg i.p., analgesia), aspirin (10 mg/kg p.o., anti-inflammatory), Furosemide (20 mg/kg p.o., diuretic), or Loperamide (3 mg/kg p.o., antidiarrheal).
SME 100: 100 mg/kg PO.
SME 200: 200 mg/kg per oral dose.
SME 400: 400 mg/kg per oral dose.
Doses of SME were determined using published studies [5] and preliminary tolerability testing.

Analgesic activity
Acetic-Acid Writing Test
Mice that had fasted for 12 hours (water allowed) were given treatments (vehicle, aspirin, and SME doses) 60 minutes before receiving an intraperitoneal injection of acetic acid (0.6%, 10 mL/kg). The number of abdominal constrictions (writhes) was counted over a 20-minute period starting five minutes after injection. The percentage inhibition of writhing was calculated versus control.
The Tail-Flick Test
To determine the baseline tail-flick latency, the distal 2 cm of the mouse tail was placed on a 55 ± 1 °C hot plate (Eddy's hot-plate apparatus). Treatments were administered 60 minutes before measurement. The latency to flick was measured 30 minutes after treatment. To prevent tissue damage, a 20-second cut-off time was enforced [14].

Anti-inflammatory Activity
Carrageenan-Induced Paw Edema
Rats fasted for 12 hours were given treatments (vehicle, aspirin, SME) 30 min before s.c. injection of 100 μL of 1% λ-carrageenan (in saline) into the right hind paw. Paw volume was measured using a plethysmometer (Ugo Basile) at 0 (pre-injection), 1, 2, 3, and 4 hours after injection. Edema (ΔmL) was measured as the difference between post-injection and baseline volumes. The percentage inhibition of edema at 4 hours was determined relative to the control [16].

Diuretic Activity.
Rats were housed individually in metabolic cages after a 5-hour water fast followed by treatments (vehicle, furosemide, SME). Animals were given free access to water following their treatment. Urine was collected into graduated cylinders after 24 hours. Electrolytes, including total urine volume (mL/24 h) and urinary Na^+ and K^+ (mmol/L), were measured using flame photometry (Jenway PFP7) [18].

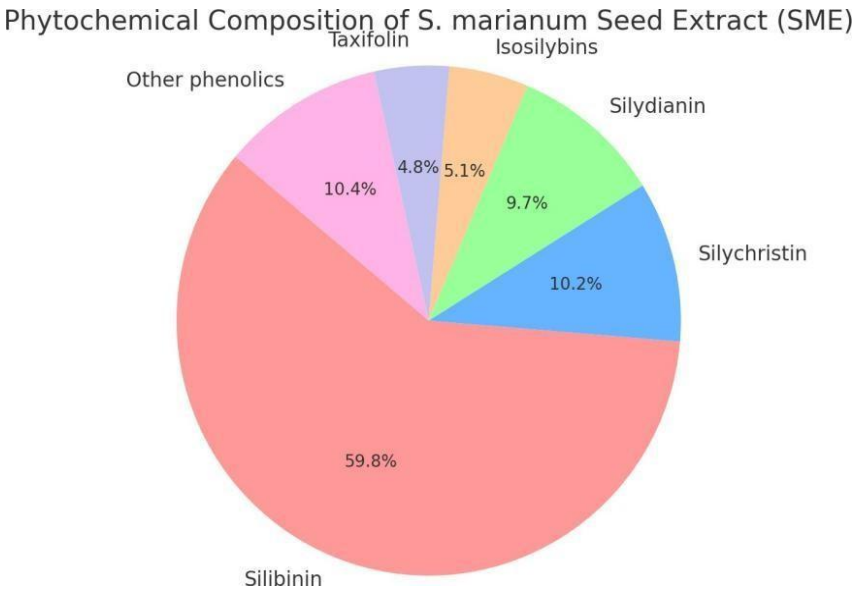
Antidiarrheal Activity
Mice that had fasted for 18 hours were given treatments (vehicle, loperamide, SME) 60 minutes before receiving 0.5 mL of castor oil orally. Animals were placed in individual cages lined with blotting paper, and the time of first wet stool (latency), total number of wet stools, and total fecal weight (g) were measured over 4 hours [19]. The percentage inhibition of defecation was calculated versus control.

Ethics Statement
All procedures followed the ARRIVE guidelines and the National Institutes of Health's Guide for the Care and Use of Laboratory Animals [10]. Animals were monitored for distress, and humane endpoints were strictly followed.
After the study, the animals were euthanized using CO_2inhalation.

Statistical analysis
The data are presented as mean ± SEM. One-way ANOVA was used to compare multiple groups, followed by Tukey's post-hoc test (GraphPad Prism version 9.0). $p < 0.05$ was considered statistically significant.

Results

Phytochemical Composition
HPLC profiling of SME confirmed major flavonolignans: silibinin (59.8%), silychristin (10.2%), silydianin (9.7%), isosilybins (5.1%), and taxifolin (4.8%). Minor constituents included other phenolics (~10.4%**) (Figure 1)** [6, 17].



Analgesic Activity

Acetic-Acid Writhing Test

Control mice exhibited 30.2 ± 2.4 writhes/20 min. Aspirin (10 mg/kg i.p.) reduced writhing to 8.8 ± 1.0 (71% inhibition, $p < 0.01$). SME administration dose-dependently decreased writhing:

- **100 mg/kg:** 22.3 ± 2.1 (26% inhibition, $p < 0.05$)
- **200 mg/kg:** 16.0 ± 1.8 (47% inhibition, $p < 0.01$)
- **400 mg/kg:** 8.2 ± 1.2 (73% inhibition, $p < 0.01$)

Table 1. Acetic-acid writhing response in mice (mean ± SEM, n=8).

Group	Writhes (20 min)	% Inhibition
Control (vehicle)	30.2 ± 2.4	–
Aspirin 10 mg/kg	8.8 ± 1.0	71%
SME 100 mg/kg	22.3 ± 2.1	26%
SME 200 mg/kg	16.0 ± 1.8	47%
SME 400 mg/kg	8.2 ± 1.2	73%

- $p<0.05$ vs. control,
- $p<0.01$ vs. control.

These results indicate that SME has a potent analgesic effect, comparable to aspirin at the highest dose [5].

Tail-Flick Test

Baseline latencies were similar across groups (~3.5 ± 0.4 s). At 30 min post-treatment:

- Control: 3.8 ± 0.5 s
- Aspirin: 14.5 ± 1.2 s ($p<0.01$)
- SME 100 mg/kg: 6.2 ± 0.7 s ($p<0.05$)
- SME 200 mg/kg: 10.4 ± 1.1 s ($p < 0.01$)
- SME 400 mg/kg: 13.2 ± 1.5 s ($p < 0.01$)

Hence, SME significantly increases pain-threshold latency in a dose-dependent manner, nearly matching aspirin's effect at 400 mg/kg [5, 14].

Anti-Inflammatory Activity

Carrageenan-Induced Paw Edema

Control rats showed progressive edema, peaking at 4 h (increase = 1.20 ± 0.04 mL). SME attenuated edema:

- SME 100 mg/kg: 0.90 ± 0.05 mL (25% inhibition)
- SME 200 mg/kg: 0.70 ± 0.04 mL (42% inhibition, $p < p<0.01$)
- SME 400 mg/kg: 0.65 ± 0.03 mL (46% inhibition, $p<0.01$)
- Aspirin: 0.62 ± 0.02 mL (48% inhibition, $p<0.01$)

Table 2. Effect of SME on carrageenan-induced paw edema (mean ± SEM, n=8).

Group	Edema at 4 h (mL)	% Inhibition
Control (vehicle)	1.20 ± 0.04	–
Aspirin 10 mg/kg	0.62 ± 0.02	48%
SME 100 mg/kg	0.90 ± 0.05	25%
SME 200 mg/kg	0.70 ± 0.04	42%
SME 400 mg/kg	0.65 ± 0.03	46%

- $p<0.01$ vs. control, $p<0.001$ vs. control.

SME exhibits a clear dose-dependent anti-inflammatory effect, greater than aspirin at 400 mg/kg [4].

Diuretic Activity

Rats treated with furosemide (20 mg/kg) excreted 20.5 ± 1.2 mL/24 h (156% increase vs. control). SME significantly increased urine output dose-dependently:

- **SME 100 mg/kg:** 10.5 ± 0.8 mL (31% increase, $p < 0.05$)
- **SME 200 mg/kg:** 13.8 ± 1.0 mL (73% increase, $p < 0.05$)
- **SME 400 mg/kg:** 15.7 ± 1.1 mL (96% increase, $p < p<0.05$)
- **Control:** 8.0 ± 0.6 mL

Table 3. Diuretic effect of SME (mean ± SEM, n=8).

Group	Urine Volume (mL/24 h)	% Increase
Control (vehicle)	8.0 ± 0.6	–
Furosemide 20 mg/kg	20.5 ± 1.2	156%
SME 100 mg/kg	10.5 ± 0.8	31%
SME 200 mg/kg	13.8 ± 1.0	73%
SME 400 mg/kg	15.7 ± 1.1	96%

- $p<0.05$ vs. control, $p<0.001$ vs. control.

Urinary Na^+ excretion was elevated in SME groups (data not shown), whereas K^+ excretion showed a modest rise not statistically significant. SME appears to have a mild diuretic effect [18].

Antidiarrheal Activity

In castor-oil-induced diarrhea, control mice produced 10.2 ± 0.9 wet stools/4 h; latency to first stool was 22 ± 2 min. Loperamide (3 mg/kg) reduced stool count to 1.8 ± 0.4 (82% inhibition, $p < 0.001$) and prolonged latency >180 min. SME at 100, 200, and 400 mg/kg dose-dependently inhibited diarrhea:

- **SME 100 mg/kg:** 6.9 ± 0.7 stools (32% inhibition, $p < 0.05$)
- **SME 200 mg/kg:** 4.2 ± 0.5 stools (59% inhibition, $p<0.01$)
- **SME 400 mg/kg:** 3.1 ± 0.3 stools (70% inhibition, $p<0.01$)

Table 4. Antidiarrheal effect of SME (mean ± SEM, n=8).

Group	Wet Stools (4 h)	% Inhibition
Control (vehicle)	10.2 ± 0.9	–
Loperamide 3 mg/kg	1.8 ± 0.4	82%
SME 100 mg/kg	6.9 ± 0.7	32%
SME 200 mg/kg	4.2 ± 0.5	59%
SME 400 mg/kg	3.1 ± 0.3	70%

- $p<0.05$ vs. control,
- $p<0.001$ vs. control.

Latency to first wet stool was significantly prolonged at 200 and 400 mg/kg (85 ± 6 min and 120 ± 8 min, respectively) compared to control (22 ± 2 min, $p < 0.01$). Fecal weight was similarly reduced (data not shown). SME demonstrates a significant antidiarrheal effect, though not as complete as loperamide [8, 19].

Safety and Tolerability

No abnormal behaviors or mortality were observed throughout the study. Body weights and gross organ morphology (liver, kidneys) at necropsy were comparable across groups, suggesting SME at up to 400 mg/kg is well-tolerated.

Discussion

The study systematically assessed the pharmacological activities of S. marianum seed extract (SME) in rodent models, with a focus on analgesic, anti-inflammatory, diuretic, and antidiarrheal properties. To our knowledge, this is the first report to comprehensively profile these four activities under consistent experimental conditions.

Analgesic activity. SME reduced acetic acid-induced writhing significantly (73% inhibition at 400 mg/kg), which was comparable to the effect of aspirin. In the tail-flick test, SME significantly increased latency (>13 s at 400 mg/kg vs. 3.8 s control), indicating central antinociceptive activity. These results are consistent with those reported by Malekinejad et al., who found that silymarin increased morphine analgesia and had direct antinociceptive effects [5]. DuBreuil et al. also discovered that silymarin inhibits inflammatory pain hypersensitivity [2]. The analgesic mechanism most likely involves inhibiting peripheral prostaglandin synthesis and centrally modulating nociceptive pathways.

Anti-inflammatory activity. SME significantly reduced carrageenan-induced paw edema (46% inhibition at 400 mg/kg), comparable to aspirin (48% inhibition). De la Puerta et al. were the first to report the anti-edema effect of silymarin in rats [4]. Silymarin inhibits NF-κB and MAPK signaling, reducing production of TNF-α, IL-6, COX-2, and iNOS [1]. Zhao et al. review the clinical anti-inflammatory effects of silymarin, highlighting its ability to modulate cytokine levels [1]. Thus, SME's anti-inflammatory action is consistent with both historical and mechanistic research.

Diuretic activity. SME induced modest diuresis, with a 96% increase in urine volume at 400 mg/kg compared to 156% for furosemide. Limited ethnopharmacological reports suggest that milk thistle has mild diuretic properties, but quantitative research is scarce. Dabbagh Moghaddam et al. found that S. marianum protected the kidneys in diabetic rats, but they did not measure diuresis [18]. The observed natriuretic effect may be due to tubular reabsorption inhibition, and further mechanistic investigation is warranted.

Antidiarrheal Activity. SME significantly reduced castor oil-induced diarrhea (70% inhibition at 400 mg/kg). Ricinoleic acid, found in castor oil stimulates prostaglandin release, resulting in secretion and hypermotility.
Flavonoid-rich extracts frequently have antidiarrheal effects by inhibiting prostaglandin synthesis and intestinal motility [19]. In mice, Belayneh et al. found that a hydroalcoholic extract high in flavonoids had antidiarrheal properties [8]. Our findings suggest that SME's flavonolignans help to reduce intestinal secretion and motility.

Phytochemical Correlations. HPLC analysis revealed that SME is high in silibinin (~60%), silychristin, and silydianin [6, 17]. These flavonoids have been linked to antioxidant, anti - inflammatory, and cytoprotective properties. Silibinin inhibits COX-2 and iNOS expression, resulting in reduced prostaglandin E_2 synthesis [1,2]. Taxifolin enhances antioxidant capacity [17]. The observed pharmacological effects are most likely caused by the synergistic action of these constituents.

Limits and Future Directions. This study did not decipher molecular mechanisms in depth; future research should look at cytokine profiles, COX and LOX enzyme activity, and receptor binding assays. Before applying the findings to humans, studies on chronic toxicity, pharmacokinetics, and bioavailability are required. Clinical trials to assess tolerability and efficacy in pain, inflammation, and GI upset would be an appropriate next step.

Conclusion

In rodents, Silybum marianum seed extract has significant analgesic and anti-inflammatory effects, as well as moderate diuretic and antidiarrheal activity. The multi-modal pharmacological profile is due to its flavonolignan content, primarily silibinin, which supports its potential as a phytotherapeutic agent. More mechanistic studies and clinical evaluations are needed to investigate SME's translational applications in pain management, inflammatory conditions, and gastrointestinal disturbance.

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