Comprehensive Journal of Science
Volume (11), Issue (42), (Sapt, 2026)
ISSN: 3104-6266

مجلة العلوم الشاملة
المجلد (11) العدد 42 سبتمبر 2026
ردمد: 3104-6266

Preliminary Evaluation of the Antinociceptive Activity of the Ethanolic Leaf Extract of *Datura metel* L. Collected in Misurata, Libya, Using the Hot Plate Test in Mice

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Received: 08/9/2026 Revised: 13/9/2026 Accepted: 05/10/2026 Publication: 05/11/2026

Abstract

Background: *Datura metel* L. (Solanaceae) is used in traditional medicine for pain relief, yet experimental data on plants growing in Libya are lacking.

Objective: To make a preliminary assessment of the antinociceptive activity of the 75% ethanolic leaf extract of *D. metel* collected in Misurata, Libya, in mice.

Methods: Dried leaf powder (35 g) was macerated in 175 mL of 75% ethanol for 72 h with continuous stirring, filtered and dried at 40 °C. Ten Swiss albino mice (n = 2 per group) were allocated to five groups: untreated control, normal saline, paracetamol (standard), and extract solutions of 10% and 20% (w/v), all given orally at 10 mL/kg. Reaction latency (paw licking, paw lifting or jumping) was recorded on a hot plate at 0, 60 and 90 min. One-way ANOVA with Tukey's post hoc test was used.

Results: At 60 min, latency differed significantly among groups ($p = 0.016$). The 10% extract produced the longest latency (10.22 ± 0.005 s) compared with control (4.03 ± 0.65 s) and saline (4.88 ± 0.08 s) (both $p < 0.05$), but not compared with paracetamol (6.83 ± 1.53 s). The 20% extract (6.06 ± 0.59 s) was not different from the vehicle. No significant differences were found at 0 min ($p = 0.111$; plate at 30 °C) or at 90 min ($p = 0.540$), where the saline group also showed prolonged latencies.

Conclusion: The 10% extract showed a short-lived, non-dose-dependent increase in hot plate latency at 60 min. Given the very small sample, the absence of motor and toxicity assessment and the high nominal dose, these findings are preliminary and hypothesis-generating only.

Keywords: *Datura metel*; antinociceptive; hot plate; tropane alkaloids; Libya; Swiss albino mice

1. Introduction

1.1 Pain and the need for safer analgesics

Pain is among the most frequent reasons for seeking medical care, and its pharmacological treatment relies mainly on two drug classes: non-steroidal anti-inflammatory drugs (NSAIDs) and opioids. Both are effective but limited. NSAIDs are associated with gastrointestinal, renal and cardiovascular adverse effects, whereas opioids cause sedation, respiratory depression, tolerance and dependence [11]. Paracetamol is widely used for mild-to-moderate pain, but its analgesic efficacy is modest and its

mechanism of action is only partly understood [12,13]. These limitations, together with the cost of imported pharmaceuticals, sustain interest in new analgesic leads, particularly from plants.

1.2 Medicinal plants as a source of analgesic drugs

Natural products have supplied or inspired a large share of the drugs in clinical use [8]. Morphine from the opium poppy, salicylates from willow bark and the tropane alkaloids atropine and scopolamine from the Solanaceae are classical examples [8,11]. In many communities, including those of North Africa and the Mediterranean region, plants are still used as a first resort for pain, spasm and inflammation, usually without dose standardisation or safety data [3]. Experimental testing of such uses in controlled animal models is the first step in separating genuine activity from folklore and in identifying toxic risk [22].

1.3 The genus *Datura* and *D. metel*

Datura L. belongs to the Solanaceae, the family that also includes tomato, potato and tobacco, and comprises about a dozen species of herbs and shrubs distributed in warm regions worldwide [3,4]. *Datura metel* L. (devil's trumpet, thorn apple) is an erect herb generally 0.5–1.5 m tall, with simple, alternate, ovate leaves that have unequal bases, large trumpet-shaped flowers up to about 20 cm long (white, yellow or purple-tinged), and globose capsules bearing many flat brown seeds [4]. The genus was long thought to have originated in the Indian subcontinent, but current evidence points to the Americas, and the plant has since become naturalised across tropical and temperate regions, including the Mediterranean basin [4]. It grows on waste ground, field margins and roadsides and is also cultivated as an ornamental.

1.4 Phytochemistry and traditional use

The pharmacological activity of *Datura* is attributed mainly to tropane alkaloids, chiefly L-hyoscyamine (which racemises to atropine during extraction and storage) and scopolamine (hyoscine) [3,9]. Their content varies between plant organs, developmental stages, seasons and growing conditions [3,9,10]. Other reported constituents include withanolides, flavonoids and phenolic compounds [3,4]. Traditional uses described in the literature include the treatment of asthma, intestinal and urinary spasm, rheumatic and muscular pain, toothache and nervous disorders [3,4,10].

1.5 Pharmacology and toxicity

Hyoscyamine, atropine and scopolamine are competitive antagonists at muscarinic acetylcholine receptors. Peripheral effects include reduced secretions, smooth-muscle relaxation, mydriasis and tachycardia, while central effects range from sedation and amnesia to agitation, hallucinations and delirium [11]. Overdose produces the anticholinergic toxidrome (dry mucosae, dilated pupils, hyperthermia, tachycardia, confusion), and accidental or intentional poisonings with *Datura* are well documented, reflecting a narrow margin between a pharmacologically active and a toxic dose [9,10]. Any claim of analgesia must therefore be considered together with dose and tolerability, because central depressant or motor effects can mimic analgesia in reflex-based tests.

1.6 Evidence for antinociceptive activity

Antinociceptive or analgesic activity has been reported in rodents for extracts of several *Datura* species and plant parts, including the aqueous extract of *D. metel* seeds [5], and has been summarised in

reviews [3,4,9,10]. These studies differ in species, plant organ, solvent, dose, route of administration and nociceptive model, sample sizes are often small, and the mechanism is unclear. The mechanistic link is also not straightforward: activation of central muscarinic receptors is antinociceptive in rodents, and muscarinic antagonists generally attenuate rather than produce this effect [23]. If an antimuscarinic-rich extract does increase reaction latency, other constituents, non-cholinergic mechanisms or non-specific effects such as sedation must be considered. To our knowledge, no experimental data exist for leaf material of *D. metel* growing in Libya.

1.7 The hot plate model of thermal nociception

The hot plate test, introduced by Woolfe and MacDonald and refined by Eddy and Leimbach, measures the latency of a mouse to show a nociceptive response (paw licking, paw lifting or jumping) when placed on a surface held at a noxious temperature, typically 50–56 °C [1,2,22]. These behaviours are integrated at supraspinal level, which makes the test sensitive to centrally acting analgesics [7]. Its main limitations are that sedation, motor impairment or hypothermia can prolong latency without true analgesia, that repeated exposure of the same animal changes the response through learning and stress, and that peripherally acting drugs such as NSAIDs are generally weak in this model [7,22]. Paracetamol, which acts partly through central mechanisms, shows only modest effects in thermal tests and is used here as a reference rather than as a strong positive control [12,13].

1.8 Rationale and aim

Datura is common around Misurata, Libya, and is used informally in traditional practice, yet no experimental evidence on its analgesic effect or its safety in the local material is available. The aim of this study was therefore to make a preliminary assessment of the antinociceptive activity of the 75% ethanolic leaf extract of *D. metel* collected in Misurata, using the hot plate test in mice. The specific objectives were (i) to describe the microscopic identification features of the leaf, (ii) to compare reaction latency after oral administration of 10% and 20% (w/v) extract with that of untreated, vehicle and paracetamol-treated mice at 60 and 90 min, and (iii) to document the behavioural responses observed, so as to guide the design of a larger, adequately powered study.

2. Materials and Methods

2.1 Plant material

Leaves of *Datura metel* L. were collected in Misurata, Libya, on [collection date]. The plant was identified by [name, affiliation], and a voucher specimen was deposited at [herbarium, voucher no.]. Leaves were washed free of dust, shade-dried at room temperature for two weeks, ground with an electric grinder and stored in airtight containers until use.

2.2 Microscopic examination

Fresh leaf pieces were cleared in 10% NaOH for 20 min, mounted in glycerin on glass slides and examined under a light microscope at ×10 and ×40 to document diagnostic features (trichomes and stomata) [20,21].

2.3 Extraction

Thirty-five grams of leaf powder were weighed on an analytical balance and macerated in 175 mL of 75% ethanol (ethanol:water) in a conical flask. The flask was placed on a hot plate with a magnetic stirrer for 72 h under continuous stirring. The mixture was filtered through filter paper, and the filtrate was dried in an oven at 40 °C until constant weight. The 75% ethanol was prepared from 96% ethanol (136.7 mL of 96% ethanol made up to 175 mL with 38.3 mL distilled water). Extraction yield: [% w/w].

2.4 Preparation of test solutions

Test solutions were prepared by dissolving 0.2 g (10% w/v) or 0.4 g (20% w/v) of dry extract in 2 mL distilled water. Each animal received a volume of 10 mL/kg body weight (0.18–0.31 mL per mouse), calculated from its body weight on the day of the test. The nominal dose therefore corresponds to approximately 1000 mg/kg (10%) and 2000 mg/kg (20%) of dry extract [authors to confirm]. A future study should select doses by stepwise acute toxicity testing [18] and express them as human-equivalent doses on a body-surface-area basis [17].

2.5 Animals and ethics

Ten adult Swiss albino mice (18.5–31.1 g) obtained from Tripoli, Libya, were housed in clean polypropylene cages with softwood bedding under controlled room conditions, with free access to standard chow and drinking water. The experiment was carried out in the pharmacognosy laboratory of the Faculty of Medical Technology, Misurata. The protocol was approved by [institutional animal ethics committee, approval no.]. Reporting follows the ARRIVE 2.0 guidelines where applicable [6].

2.6 Experimental groups

The ten mice were allocated to five groups (n = 2 each) [method of allocation]: (1) untreated control; (2) vehicle (0.9% normal saline); (3) standard, paracetamol ([dose and unit as recorded: 0.20 mg/kg; confirm]); (4) extract 10% w/v; (5) extract 20% w/v. All treatments were given orally by gavage at 10 mL/kg.

2.7 Hot plate test

After dosing, each mouse was placed on an electrically heated hot plate (display accuracy 0.1 °C) enclosed by a transparent cylinder. Reaction latency was defined as the time from placement on the plate to the first nociceptive response (paw licking, paw lifting or jumping), measured with a stopwatch at 0, 60 and 90 min after dosing. At 0 min the plate temperature recorded was 30 °C, and at 60 and 90 min it was 60 °C. The cut-off time was [x s]. The same animals were tested at all three time points. The observer was [blinded / not blinded].

2.8 Statistical analysis

Latency is expressed as mean ± SEM. Groups were compared at each time point by one-way ANOVA followed by Tukey's multiple comparison test [14] in SPSS; $p < 0.05$ was considered significant. Because each group contained two animals, the analysis is exploratory [15,16].

3. Results

3.1 Plant material and extract

Microscopic examination of cleared leaf fragments showed the epidermal trichomes and stomata used for identification (Figures [3-4 and 3-5 of the original work]). The dried ethanolic extract was dark green; extraction yield: [% w/w]. The 10% and 20% (w/v) test solutions were prepared from 0.2 g and 0.4 g of dry extract in 2 mL distilled water, respectively.

3.2 Animals and administered volumes

Ten mice (two per group) weighing 18.5–31.1 g were used. Mean body weight ranged from 22.2 g in the 20% extract group to 29.0 g in the 10% extract group (Table 1). Volumes were calculated at 10 mL/kg for each animal.

Table 1. Body weight and administered volume of each mouse (A, B).

Group	Weight A (g)	Weight B (g)	Mean weight (g)	Volume A (mL)	Volume B (mL)
Control	22.5	23.2	22.9	0.23	0.23
Normal saline	28.5	27.1	27.8	0.28	0.27
Paracetamol	22.2	27.3	24.8	0.20	0.20
Extract 10%	26.8	31.1	29.0	0.27	0.31
Extract 20%	18.5	25.8	22.2	0.18	0.26

The volumes of 0.20 mL recorded for both paracetamol animals are as logged in the original records [confirm].

3.3 Individual reaction latencies

Individual latencies are listed in Table 2 to show the spread within each group, which is relevant given n = 2.

Table 2. Individual hot plate latencies (s).

Group	0 min A	0 min B	60 min A	60 min B	90 min A	90 min B
Control	38.35	32.40	4.68	3.38	4.22	4.68
Normal saline	44.10	35.28	4.96	4.80	10.18	14.00
Paracetamol	50.40	35.50	5.29	8.36	13.23	11.44
Extract 10%	30.00	48.50	10.21	10.22	13.30	11.66
Extract 20%	77.80	58.50	6.65	5.47	27.17	6.34

At 60 min both animals given the 10% extract responded after about 10.2 s, longer than any animal in the control, saline or 20% extract groups (3.4–6.7 s) and than one of the two paracetamol animals (5.3 s; the other responded at 8.4 s). At 90 min the 20% extract group contained both the longest latency of the experiment at that time point (27.17 s) and one of the shortest among treated animals (6.34 s).

3.4 Group means

Table 3. Reaction latency (s) in the hot plate test (mean ± SEM, n = 2 per group). SEM was recalculated from the individual values in Table 2.

Group	0 min (plate 30 °C)	60 min (plate 60 °C)	90 min (plate 60 °C)
Control	35.38 ± 2.97	4.03 ± 0.65	4.45 ± 0.23
Normal saline	39.69 ± 4.41	4.88 ± 0.08	12.09 ± 1.91
Paracetamol	42.95 ± 7.45	6.83 ± 1.53	12.34 ± 0.90
Extract 10%	39.25 ± 9.25	10.22 ± 0.005	12.48 ± 0.82
Extract 20%	68.15 ± 9.65	6.06 ± 0.59	16.76 ± 10.42
ANOVA p	0.111	**0.016**	0.540

At 0 min the plate was at 30 °C, a non-noxious temperature, so these values are reference readings and were not interpreted as antinociception. The apparently longer latency of the 20% group at that time (68.15 s) was not significant (p = 0.111).

3.5 Statistical comparisons

One-way ANOVA (4 and 5 degrees of freedom) was significant only at 60 min, where group allocation accounted for about 88% of the total variance (Table 4).

Table 4. One-way ANOVA of latency at each time point.

Time point	F(4,5)	p	η^2
0 min	3.31	0.111	0.73
60 min	9.09	0.016	0.88
90 min	0.87	0.540	0.41

Tukey's test at 60 min (honestly significant difference = 4.13 s, computed from the pooled within-group variance) showed that the 10% extract differed from the control (mean difference 6.19 s) and saline (5.34 s) groups at $p < 0.05$ (Table 5). It did not differ from paracetamol (3.39 s). The difference between the 10% and 20% extracts (4.16 s) was marginal, just above the critical value. No other pair differed.

Table 5. Pairwise mean differences in latency at 60 min (s); * = exceeds the Tukey critical value of 4.13 s.

Comparison	Mean difference (s)
Extract 10% vs control	6.19 *
Extract 10% vs saline	5.34 *
Extract 10% vs extract 20%	4.16 (marginal)
Extract 10% vs paracetamol	3.39
Paracetamol vs control	2.80
Extract 20% vs control	2.03
Paracetamol vs saline	1.95
Extract 20% vs saline	1.18
Saline vs control	0.85

3.6 Effect relative to the vehicle and time course

Relative to the saline group, the mean latency at 60 min was 109% longer with the 10% extract, 40% longer with paracetamol and 24% longer with the 20% extract, whereas the untreated control was 17% shorter. At 90 min these relative differences had disappeared: the 10% extract and paracetamol were within 3% of the saline group, and the 20% extract was 39% longer but with a very wide spread (Table 6).

Table 6. Change in mean latency between 60 and 90 min.

Group	60 min (s)	90 min (s)	Change
Control	4.03	4.45	+10%
Normal saline	4.88	12.09	+148%
Paracetamol	6.83	12.34	+81%
Extract 10%	10.22	12.48	+22%
Extract 20%	6.06	16.76	+177%

All groups except the untreated control showed longer latencies at 90 than at 60 min, including the saline group, in which latency rose by about 7 s.

3.7 Behavioural responses

The nociceptive behaviour that ended each trial was recorded for each animal (Table 7). At 60 min licking of the hind paws was the usual endpoint in all treated groups, whereas the saline animals showed only general movement. At 90 min the endpoint in the saline, paracetamol and 10% extract groups was

jumping in all animals, while control animals responded by lifting the paws. The behaviours recorded at 0 min (mostly licking) occurred on a plate at 30 °C and cannot be regarded as nociceptive.

Table 7. Endpoint behaviour of each animal (A / B).

Group	0 min (30 °C)	60 min	90 min
Control	lick / lick	lick / paw lift	paw lift / paw lift
Normal saline	lick / lick	movement / movement	jump / jump
Paracetamol	paw lick / paw lick	lick / paw lick	jump / jump
Extract 10%	paw lift / jump	paw lick / lick	jump / jump
Extract 20%	paw lick / paw lick	paw lick / lick	jump / paw lick

4. Discussion

The main finding is a significant between-group difference at 60 min, driven by the 10% extract, whose latency was about twice that of vehicle and 2.5 times that of untreated animals. The effect was not larger with the 20% extract, so no dose–response relationship can be claimed, and it was not present at 90 min. A non-monotonic response could reflect true pharmacology (for example, central side effects of the anticholinergic alkaloids at the higher dose), but with two animals per group it cannot be distinguished from individual variation, as the wide SEM of the 20% group at 90 min illustrates.

Several features of the design limit interpretation. First, the same mice were placed on the hot plate three times, and the saline group's latency rose from 4.88 s to 12.09 s between 60 and 90 min, matching paracetamol and the 10% extract at 90 min. Repeated exposure, handling and stress-induced analgesia are likely contributors, and the lack of separation from the vehicle at 90 min does not support a sustained effect. Second, the 0-min measurements were taken at a plate temperature that does not elicit nociceptive behaviour, and an effect within minutes of oral dosing would in any case be pharmacokinetically unlikely. Third, in the hot plate test, sedation, motor impairment or altered thermoregulation can lengthen latency independently of analgesia; muscarinic antagonists such as hyoscyamine, atropine and scopolamine are known to cause such effects [3,4], and no motor coordination or locomotor test was run. Fourth, the nominal doses were high (about 1000–2000 mg/kg), a range in which toxicity of a *Datura* extract is a concern.

The extract showed no chemical profile in this study, so attributing the effect to tropane alkaloids, or to any interaction with opioid receptors, is speculative. Muscarinic mechanisms are the more plausible candidates [3,4]. The activity of the 10% extract was not statistically different from paracetamol, a drug that is itself only weakly active in thermal tests, so "comparable to paracetamol" should not be read as evidence of clinical-grade analgesia. Previous reports of analgesic activity from other parts of *D. metel*, such as the seeds [5], are consistent with, but cannot confirm, the present observation.

Limitations

The study is limited by (i) n = 2 per group and multiple repeated measures on the same animals; (ii) no phytochemical profile (alkaloid content), yield or extract standardisation; (iii) no motor/sedation or

acute toxicity assessment; (iv) a mixed endpoint (licking, lifting and jumping) and no stated cut-off; (v) no dose-ranging; (vi) a single plant collection.

5. Conclusion

In this preliminary experiment, the ethanolic leaf extract of Libyan *Datura metel* at 10% w/v increased hot plate latency in mice at 60 min compared with control and vehicle, without a dose-dependent pattern or a sustained effect at 90 min. The results justify a properly powered study designed according to the 3Rs principles [19] with dose ranging well below the present nominal doses, alkaloid quantification, a naive-animal (independent groups) or crossover design, motor and toxicity assessment, and additional nociception models.

Declarations

Ethics approval: [committee and approval number]. **Conflict of interest:** The authors declare none [confirm]. **Funding:** [none / source]. **Author contributions:** [CRediT roles]. **Data availability:** Individual animal data are provided in Table S1 [to be attached].

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