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An Analysis of Secondary Metabolites and Anesthetic Potential of Datura Flower Extract (*Datura metel L.*)

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Abstract | Datura (*Datura metel L.*) is a medicinal plant with promising potential as an anesthetic agent. Phytochemical analyses have revealed that its leaves, seeds, and flowers contain specific bioactive compounds, particularly tropane alkaloids such as scopolamine and atropine, as well as flavonoids known for their pharmacological properties. These substances contribute to the plant's sedative and anesthetic effects, making it a valuable subject for further biomedical research. This study aims to evaluate the anesthetic potential of 95% methanol extract of Datura flower (*Datura metel L.*), using a maceration method to obtain the liquid extract. The extract was administered orally to white rats (*Rattus norvegicus*) of varying sex, body weight (150–250 g), and age (2–3 months). The subjects were divided into two groups: a control group that received ketamine HCl (80 mg/kg BW intramuscularly) and a treatment group that received Datura flower extract at graded doses of 100, 300, 500 and 700 mg/kg BW. Phytochemical screening revealed the presence of alkaloids, saponins, and tannins, which are secondary metabolites potentially responsible for anesthetic activity. The acute toxicity test conducted using OECD Guideline 425 determined an LD_{50} >2000 mg/kg BW, classifying the extract as practically non-toxic. Clinical observations indicated that the extract produced strong analgesic effects and mild muscle relaxation, but no observable sedative effect, as assessed through the anesthesia triad: analgesia, muscle relaxation, and hypnosis. Although treatment groups tended to show shorter induction times and durations compared to the ketamine group, these differences were not statistically significant. Moreover, the duration of the analgesic and relaxant responses induced by *Datura metel* extract did not follow a dose-dependent trend. Hematological parameters including hemoglobin (Hb), hematocrit (Ht), erythrocyte, and leukocyte counts remained within the normal physiological range across all administered doses. Statistical analysis showed no significant differences ($p > 0.05$) between the control and treatment groups. The coefficient of variation (%CV) for hemoglobin, hematocrit, and erythrocyte counts across treatment groups ranged from 5.2% to 9.8%, indicating stable hematological responses. Histopathological analysis of brain tissues revealed no significant structural alterations, supporting the safety of the extract at tested doses. These findings suggest that methanol extract of *Datura metel L.* possesses potential anesthetic properties, particularly as an analgesic and muscle relaxant, with a favorable safety profile.

Keywords | Anesthesia, Datura metel, Hematological Profile, Brain Histopathology, Secondary Metabolites, Acute Toxicity

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INTRODUCTION

Nature-based therapies encompass a range of therapeutic modalities that incorporate nature as a fundamental element of the therapeutic process (Naor and Mayseless, 2020). The utilization of herbal medicine as an adjunct therapy is expanding swiftly within healthcare. This may occur due to numerous individuals seeking complementary or combination therapies to alleviate severity and minimize negative effects. In Indonesia, herbal therapy is the most preferred complementary treatment among the people, particularly due to the accessibility of herbal medications. *Datura metel L.* is recognized for its anesthetic potential due to the presence of specific secondary metabolites, tropane alkaloids (e.g., scopolamine, hyoscyamine, and atropine), and flavonoids, tannins, and saponins, which have been identified through qualitative phytochemical screening. These compounds are known to exert analgesic, anticholinergic, and muscle relaxant effects, supporting the traditional use of *Datura* in local anesthesia.

Datura has been utilized as traditional medicine for generations, and it is empirically employed as an antibacterial, antiseptic, narcotic, and sedative treatment (Carpa *et al.*, 2017; Suryadi *et al.*, 2021). Various parts of the plant including seeds, flowers, and leaves contain bioactive compounds that contribute to its pharmacological effects (Ganesh *et al.*, 2015; Samuel *et al.*, 2018). Phytochemical analyses have identified the presence of steroids, flavonoids, phenols, tannins, saponins, and alkaloids, which are responsible for these bioactivities (Samuel *et al.*, 2018). Experimental applications have demonstrated its anesthetic potential across species. A 10% ethanol extract from *Datura* seeds has been used as an anesthetic agent in Kintamani dogs (Sukariada *et al.*, 2016; Sudira *et al.*, 2024), while analgesic effects have been observed in male Wistar rats (Gente et al., 2015). Additionally, *Datura Metel L.* Seed Extract have been applied as an anesthetic in *Epinephelus sp.* fish grouper transport to reduce stress responses in immobilization (Saputra *et al.*, 2021).

Datura metel L. has been found to be pharmacologically important species because of its different pharmacological and traditional uses, such as hepatoprotective, antiviral effect, antibacterial effect, anti-asthmatic, analgesic, antipyretic, nephroprotective effect, anticancer, and antifungal effect (Waqas *et al.*, 2021). Recent studies have confirmed that leaves and bulbs of *Datura metel L.* contain various secondary metabolites, including alkaloids, flavonoids, saponins, phenolic compounds, and tannins, which contribute to its pharmacological activities (Archana *et al.*, 2023; Mbaye *et al.*, 2025). The flowers contain larger quantities of alkaloid, tannin, and saponin. The alkaloids present, such as atropine, scopolamine, and hyoscyamine, are known for their anticholinergic and analgesic effects, which contribute significantly to anesthetic mechanisms (Sholichah *et al.*, 2017; Tamalawe *et al.*, 2021). These compounds' presence in *D. metel* flowers thus underscores their potential utility as anesthetic agents.

An ideal anesthetic should be able to induce analgesia, sedation, muscle relaxation, unconsciousness, and maintain homeostasis of vital systems, considering economic feasibility and practical application (Pemayun and Sudisma, 2018; Prihatiningsih *et al.*, 2022). However, according to Sudisma *et al.* (2023), no single anesthetic compound has yet been developed that fully satisfies all these criteria. In the context of herbal-based anesthesia, rigorous evaluation of safety and efficacy is essential, including specific phytochemical profiling to identify active secondary metabolites such as tropane alkaloids (atropine, scopolamine, hyoscyamine), flavonoids, saponins, and tannins, which are known to influence central nervous system activity (Armansyah *et al.*, 2016; Hasen and Hashim, 2021). For this reason, the current study aims to assess the anesthetic properties of Datura flowers based on phytochemical analysis and other measurements of toxicity, analgesic response, sedation, relaxation, and histopathology.

MATERIAL AND METHODS

Ethical Consideration

All procedures involving test animals were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University (Approval Letter No. B/247/UN14.2.9/PT.01.04/2024).

Secondary Metabolite Test

Datura metel L. flowers were harvested in late March – mid April in period of the year 2020 from Bedugul, North of Bali, Indonesia (8°16'33.7"S 115°09'56.2"E) served as the primary experimental material. *Datura metel L.* flowers were washed and dried for seven days to produce simplicia. The dried material was ground into powder, and 200 grams of simplicia underwent extraction using the maceration method with 2 litres of 95% methanol (10% w/v). The maceration mixture was stirred periodically for over 24 hours and then filtered. The resulting macerate was evaporated using an evaporator to obtain crude extract. A 100 mg sample of crude extract was homogenized with 20 ml of 95% methanol (0.5 % w/v) for 10 minutes using a centrifuge. Extraction employs a solution based on the solubility of components in relation to one another or their polarity within the mixture. The 95% methanol is commonly used in antioxidant extraction because its polarity closely matches, making it easy to dissolve (Al-Huqail et al., 2018; Savitri *et al.*, 2022). Phytochemical tests were conducted on

the homogenized extract to identify alkaloids, flavonoids, saponins, tannins, phenols, and triterpenoid/steroid compounds.

Alkaloid Identification

One gram of the extract was placed in a test tube, treated with 2N HCl, and subsequently distributed into many test tubes. Each tube was supplemented with each reagent. If the extract constitutes a white or yellow precipitate forms upon the addition of Mayer's reagent, the extract includes alkaloids. The extract includes alkaloids if an orange precipitate is produced following the use of Dragendorff's reagent.

Flavonoid Identification

A variety of extracts were placed into a test tube; thereafter, 5 ml of hot water was added, heated for 5 minutes, and then filtered. Five millilitres of the filtrate were extracted and combined with magnesium powder, followed by the addition of one millilitre of strong hydrochloric acid to amyl alcohol; the mixture was agitated until separation occurred. The formation of an orange, red, or yellow precipitate indicates a positive presence of flavonoids.

Saponin Identification

A variety of extracts were placed into a test tube, followed by the addition of 10 ml of boiling water, which was subsequently cooled, and then the mixture was shaken rapidly for 10 seconds. A positive result for saponins is indicated by the formation of foam of 1–10 cm in height for at least 10 minutes, and the foam remains stable upon the addition of 1 drop of 1% HCl.

Tannin Identification

One gram of homogenized crude extract was put into a test tube, then one drop of Pb Acetate was added. Positive tannin results are indicated by sediment formation in the test sample.

Phenols Identification

One gram of crude extract has been homogenized and put into a test tube. Add 10 drops of 10% FeCl3 to the test tube, then observe the color change in the test sample. A change in color to blackish green or blue green indicates positive results for phenol compounds.

Steroid and Triterpenoid Identification

The homogenized crude extract was taken as much as 1 ml each and put into two different test tubes. Then, 5 ml of chloroform, 2 ml of sulfuric acid, and 2 ml of anhydrous acetic acid were added. The sample was left for five minutes to see the color change. A red or purple color change indicates positive triterpenoid results, while positive steroid results will produce a green-blue color change.

Toxicity Test

The toxicity test followed the OECD *(Organization for Economic Cooperation and Development)* 425 method, utilizing female mice (*Mus musculus*) aged 8–12 weeks and weighing 20–30 grams. Guideline 425 explains that females are typically used. This is because literature surveys of conventional LD50 tests demonstrate that there is usually little difference in sensitivity between the sexes; however, in cases where differences are observed, females are generally slightly more sensitive. Mice were divided into control and treatment groups, with 5–12 test animals randomly selected. Datura flower extract was first administered at 175 mg/kg (sublethal dose) to one test animal. Dosages were adjusted based on survival or mortality outcomes within 48 hours in the range 1.75, 5.5, 17.5, 55, 175, 550, and 2000 mg/kg following OECD 425 guidelines (OECD, 2008), based on the principle of logarithmic progression with a multiple factor of 3.2 (1/2 log unit), as follows:

$$Dose_{(n)+1} = Dose_{(n)} \times 3.2$$

Toxicity observations included pyrexia, convulsions, tremors, pain, grooming behaviour, auricular reflexes, salivation, lacrimation, hyperactivity, and mortality.

Anesthetic Triad Response Test

Twenty-five male Sprague-Dawley rats (*Rattus norvegicus*), aged 6–8 weeks and weighing 150–200 grams, were obtained from the Rat Breeding Centre, Udayana University. Controlling for type, sex, weight, and age ensures that the independent variables solely affect the research results. The experimental design employed a balanced approach to treatment administration and ease of behavioral observation, thereby minimizing the impact of other variables. The rats were randomly assigned to five groups. The control group (P0) received intramuscular ketamine HCl 100 mg/ml at a dose of 80 mg/kg, while the treatment groups (P1, P2, P3, and P4) were administered *Datura metel* flower extract orally at doses of 100, 300, 500, and 700 mg/kg, respectively. The dosage is based on the herbal ingredient's toxicity limits, rat's stomach capacity, preliminary tests, and previous anesthetic research on *Datura metel L.* (e.g., leaves and seeds). Observations focused on induction time and anesthetic duration, which were evaluated through the anesthetic triad response.

The anesthetic triad response encompassed three key indicators: analgesia, sedation, and relaxation. Analgesia was evaluated by applying a firm but non-traumatic pinch using blunt surgical forceps to the interdigital spaces of the hind limbs, the base of the tail, and the ear pinnae. The absence of a withdrawal reflex or vocalization was considered a positive indication of analgesia, as adapted from standard nociceptive response assays (OECD 420/425 guidelines). Se-

dation was assessed via three indicators: (1) pupil response to light (pupillary light reflex), (2) palpebral reflex (elicited by gently tapping the medial canthus with a cotton swab), and (3) behavioral observation of immobility and loss of righting reflex. Sedation was considered adequate when pupils were dilated, the palpebral reflex was absent, and the animal exhibited unresponsiveness to moderate tactile stimuli for ≥30 seconds. Relaxation determined by observing passive resistance in the jaw, limbs, and anal sphincter. Relaxation was confirmed when: (1) the jaw could be gently opened without resistance, (2) the limbs exhibited flaccidity upon gentle extension, and (3) the anal sphincter appeared loose and non-contractile under light digital palpation. All assessments were performed at 5-minute intervals for up to 30 minutes post-anesthetic administration.

Hematology Test

Blood samples were collected 24 hours post-administration of *Datura* flower extract. The samples were attained from each ocular vein with sterile heparin microhematocrit and placed into sterilized vials containing ethylene diamine tetra acetic acid (EDTA) for the hematology, tests were performed using a Sysmex XS-800i hematology analyzer at the UPT Provincial Health Laboratory of Bali. Blood samples collected from all animals were analyzed to determine the hemoglobin, hematocrit, erythrocytes, leukocytes, and monocytes.

Histopathological Examination

Rats were euthanized 24 hours post-treatment via cervical dislocation, followed by necropsy to collect brain samples. Brain samples were fixed in 10% neutral buffered formalin for 24 hours followed by dehydration and clearing processes. The process was carried out in one solution session using 70% ethanol, 80% ethanol, 95% ethanol, absolute ethanol, and xylene. The brain samples were then processed into paraffin-embedded tissue blocks. Three micrometer sections were deparaffinized using xylene and rehydrated with ethanol. The rehydration process was carried out with one solution session consisting of absolute ethanol, 95% ethanol, 80% ethanol, and 70% ethanol. Then, tissue staining was performed with routine Hematoxylin Eosin (HE) staining. The examination was carried out under a bright-field microscope on five fields of view at magnifications of ×100 and ×400. The histopathological changes observed were congestive lesions and perivascular edema.

Data Analysis

Quantitative response data related to mortality or other binary outcomes were analyzed using probit analysis to determine the median effective dose (ED_{50}) or median lethal dose (LD_{50}). The analysis was based on the proportion of animals responding at each dose level, with dose values transformed into logarithmic scale and response rates converted into probit units. Linear regression was then applied to estimate the dose corresponding to a 50% response. Qualitative observational data were presented descriptively. For scoring data (ordinal outcomes), statistical analysis was conducted using the Kruskal-Wallis test, followed by the Mann-Whitney U test for pairwise post hoc comparisons. All statistical analyses were performed using SPSS for Windows (version 25), and results were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Results

Phytochemical test: Phytochemical screening of the 95% methanol extract of *Datura metel L.* flowers demonstrated the presence of several secondary metabolites, including alkaloids, saponins, and tannins, as evidenced by positive results in standard qualitative tests. However, the same extract tested negative for phenolic compounds and for triterpenoids or steroids, indicating their absence or presence below detectable levels under the applied analytical conditions, as shown in Table 1.

Table 1: Phytochemical test results of the 95% methanol extract of *datura* flowers.

Phytochemical Test	Reagent	Result	Note
Alkaloid	Dragendorff's Reagent, Bouchardat's Reagent, Mayer's Reagent, Wagner's Reagent	Precipitate formed	Positive
Flavonoid	Acetone + Boric Acid + Oxalic Acid + Ether	No color change	Negative
Saponin	Distilled water, heated, shaken	Stable foam formed	Positive
Tannin	Lead (Pb) Acetate	Precipitate formed	Positive
Phenol	FeCl3 10%	No color change	Negative
Triterpenoid/ Steroid	Liebermann-Burchard	No color change	Negative

Note: Positive: contains secondary metabolite compounds; **Negative:** does not contain secondary metabolite compounds.

Toxicity test: Toxicity evaluation conducted according to OECD Guideline 425, and analyzed using probit analysis, determined that signs of toxicity were observed at doses of 550 mg/kg and 2000 mg/kg, as indicated by diminished ear reflex responses in mice. Despite these signs, no behavioral abnormalities were detected in any of the test animals, and no mortality was recorded in either the treatment or control groups throughout the 14-days observation period. The results of toxicity indicators are detailed in Table 2.

Table 2: Observation of toxicity signs in mice (*Mus musculus*).

	30 min				4 hours				24 hours			
Parameter												
	K	P1	P2	P3	K	P1	P2	P3	K	P1	P2	P3
Piloerection	x	x	x	x	x	x	x	x	x	x	x	x
Convulsions	x	x	x	x	x	x	x	x	x	x	x	x
Tremor	x	x	x	x	x	x	x	x	x	x	x	x
Pain	x	x	x	x	x	x	x	x	x	x	x	x
Grooming Behavior	N	N	N	N	N	N	N	N	N	N	N	N
Auricular Reflex (Ear Reflex)	N	N	✓	✓	N	N	N	N	N	N	N	N
Salivation	x	x	x	x	x	x	x	x	x	x	x	x
Lacrimation	x	x	x	x	x	x	x	x	x	x	x	x
Hyperactivity	x	x	x	x	x	x	x	x	x	x	x	x
Mortality	x	x	x	x	x	x	x	x	x	x	x	x
		48 hours				1 week				2 weeks		
Parameter												
	K	P1	P2	P3	K	P1	P2	P3	K	P1	P2	P3
Piloerection	x	x	x	x	x	x	x	x	x	x	x	x
Convulsions	x	x	x	x	x	x	x	x	x	x	x	x
Tremor	x	x	x	x	x	x	x	x	x	x	x	x
Pain	x	x	x	x	x	x	x	x	x	x	x	x
Grooming Behavior	N	N	N	N	N	N	N	N	N	N	N	N
Auricular Reflex (Ear Reflex)	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	x	x	x	x	x	x	x	x	x	x	x	x
Lacrimation	x	x	x	x	x	x	x	x	x	x	x	x
Hyperactivity	x	x	x	x	x	x	x	x	x	x	x	x
Mortality	x	x	x	x	x	x	x	x	x	x	x	x

Description: K = Control (distilled water 0.6 ml); P1 (175 mg/kg); P2 (550 mg/kg); P3 (2000 mg/kg). N = Normal; (x) = Did not occur; (✓) = Occurred.

Anesthetic triad response test: Anesthetic triad responses were assessed, with findings presented in Table 3. Analgesic and muscle relaxation responses at the anal sphincter showed statistically significant differences ($P < 0.05$) between the control group administered ketamine and the treatment groups receiving *Datura metel* flower extract. These findings indicate that the extract was capable of inducing measurable analgesic and relaxant effects compared to baseline. However, no significant differences ($P > 0.05$) were observed among the treatment groups themselves, regardless of dosage level. In contrast, other measured parameters, such as induction time, duration of the palpebral reflex, sleeping behavior, pupil reflex, and relaxation of the jaw and limbs, did not differ significantly ($P > 0.05$) between the ketamine control group and the *Datura metel*-treated groups. Although treatment groups tended to show shorter induction times and durations compared to the ketamine group, these differences were not statistically significant. The results indicate that strong analgesic values were observed in each individual across all indicators, with mild relaxation noted due to reduced reactivity and postural tone, as evidenced by only one indicator showing effects in the experimental observations. Moreover, the duration of the analgesic and relaxant responses induced by *Datura metel* extract did not follow a dose-dependent trend. Higher doses did not result in a proportional increase in the duration of analgesia or muscle relaxation, suggesting a plateau effect or a non-linear pharmacodynamic relationship at the tested doses.

Table 3: Mean ± standard deviation (SD) of induction time and duration of analgesia, sedative, and relaxation responses in sprague dawley rats administered *Datura* flower extract at varying doses.

Parameter	Treatment	Time (Minutes)	
		Induction	Duration
Ear Analgesics	P0	5 ± 0.00	140 ± 32.404[a]
	P1	7 ± 2.739	97 ± 45.908[a]
	P2	17 ± 14.405	48 ± 10.368[a]
	P3	19 ± 12.942	44 ± 33.615[a]
	P4	14 ± 8.944	59 ± 26.552[a]
Tail Analgesics	P0	5 ± 0.00	154 ± 44.637[a]
	P1	5 ± 0.00	114 ± 35.426[a]
	P2	5 ± 0.00	94 ± 13.416[a]
	P3	5 ± 0.00	66 ± 20.433[a]
	P4	5 ± 0.00	96 ± 23.022[a]
Interdigital Analgesics	P0	5 ± 0.00	101 ± 18.166[a]
	P1	12 ± 8.380	61 ± 14.748[a]
	P2	16 ± 21.909	62 ± 28.636[a]
	P3	20 ± 12.748	20 ± 10.00[a]
	P4	10 ± 5.00	61 ± 21.036[a]
Palpebrae Reflex Sedation	P0 – P4	ND	ND
Sleep Reflex Response	P0 – P4	ND	ND
Pupillary Reflex Sedation	P0 – P4	ND	ND
Anal Sphincter Relaxation	P0	5 ± 0.00	97 ± 52.631
	P1	13 ± 15.248	92 ± 55.857
	P2	8 ± 6.708	72 ± 2.739
	P3	10 ± 3.536	137 ± 13.509
	P4	12 ± 13.038	113 ± 13.038
Jaw Relaxation	P0 – P4	ND	ND
Locomotor Relaxation	P0	6 ± 6.519	28 ± 27.749
	P1 - P4	ND	ND

Note: P0 (Ketamine 80 mg/kg), P1 (100 mg/kg), P2 (300 mg/kg), P3 (500 mg/kg), P4 (700 mg/kg). ND = No Data; a,b, and c values with distinct alphabetic superscripts within a row exhibit significant differences ($P<0.05$).

Table 4: Mean ± SD values of hemoglobin (HB), hematocrit (HCT), erythrocytes (RBC), and leukocytes (WBC) in white rats.

Parameter				
Group	Hb (g/dL)	HCT (%)	RBC (106/µL)	WBC (103/µL)
P0	15,70± 0,55^{cd}	45,82± 1,24^{b}	9,19 ± 0,42^{bc}	12,91 ± 1,19^{ab}
P1	15,36± 1,00^{bc}	42,90± 3,04^{ab}	9,56 ± 0,46^{c}	16,97 ± 4,44^{bc}
P2	14,12± 0,92^{ab}	40,82± 2,84^{a}	8,49 ± 0,47^{ab}	14,04 ± 2,82^{b}
P3	14,30± 0,53^{ab}	41,70± 1,19^{a}	8,46 ± 0,53^{a}	14,15 ± 2,63^{b}
P4	16,68± 0,83^{d}	50,04± 1,87^{c}	9,63 ± 0,54^{c}	9,04 ± 2,08^{a}

Note: P0 (Ketamine, 80 mg/kg), P1 (100 mg/kg), P2 (300 mg/kg), P3 (500 mg/kg), P4 (700 mg/kg). Differing superscript letters in the same column indicate significant differences among treatment groups (P<0.05).

Hematology test: Hematological parameters, summarised in Table 4, displayed dose-dependent variations. Hematocrit (HCT) values at doses of 100mg/kgBW (42.90 ± 3.04%), 300mg/kgBW (40.82 ± 2.84%) and 500mg/kgBW (41.70 ± 1.19%) showed an insignificant increase over the control group (45.82 ± 1.24%), while the dose of 700mg/kgBW (50.04 ± 1.87%) showed a significant increase over the control. The erythrocyte (RBC) value at a dose of 100mg/kgBW (9.56 ± 0.46 x106 /µL) and 700mg/kgBW (9.63 ± 0.54 x106 /µL) showed an insignificant increase in the number of erythrocytes, while the dose of 300mg/kgBW (8.49 ± 0.47 x106 /µL) and 500mg/kgBW (8.46 ± 0.53 x106 /µL) showed an insignificant decrease in the number of erythrocytes compared to the control (9.19 ± 0.42 x106 /µL). The value of the number of leukocytes (WBC) at a dose of 100 mg/kgBW (16.97 ± 4.44 x103 /µL), 300 mg/kgBW (14.04 ± 2.82 x103 /µL), 500 mg/kgBW (14.15 ± 2.63 x103 /µL) and 700 mg/kgBW (9.04 ± 2.08 x103 /µL) showed a non-significant decrease compared to the control (12.91 ± 1.19 x103 /µL).

Histopathological examination: The results of histological examination of the white rat brain found changes in congestion and perivascular edema in all treatment groups. Changes in the histopathological picture of the white rat brain were categorized into scores 1 and 2, namely mild and moderate. Congestion changes in the control group were mild, while for the dose treatment group they were mild and moderate. The result illustrated in Figure 1. Statistical scoring of these lesions revealed no significant differences ($P > 0.05$) between the control group and treatment groups.

Discussion

The detection of alkaloids, saponins, and tannins in the flowers of *Datura metel L.* suggests a promising potential for their application in anesthetic formulations. These secondary metabolites are known to possess various pharmacological activities that contribute to anesthetic effects. Alkaloids, in particular, have been widely recognized for their dual role as anesthetic agents and central nervous system (CNS) stimulants (Madziga et al., 2010; Waqas *et al.*, 2021). Several alkaloid compounds, such as morphine, caffeine, cocaine, and atropine, exhibit strong analgesic properties, with effects potent enough to significantly reduce or even eliminate pain perception (Variani *et al.*, 2021). The presence of these or similar bioactive alkaloids in *Datura metel* supports its potential for pain modulation. In addition, saponins identified in the extract are reported to exert CNS-depressant effects, which may contribute to anesthesia by reducing neuronal excitability and suppressing respiratory activity (Faqihudin *et al.*, 2023).

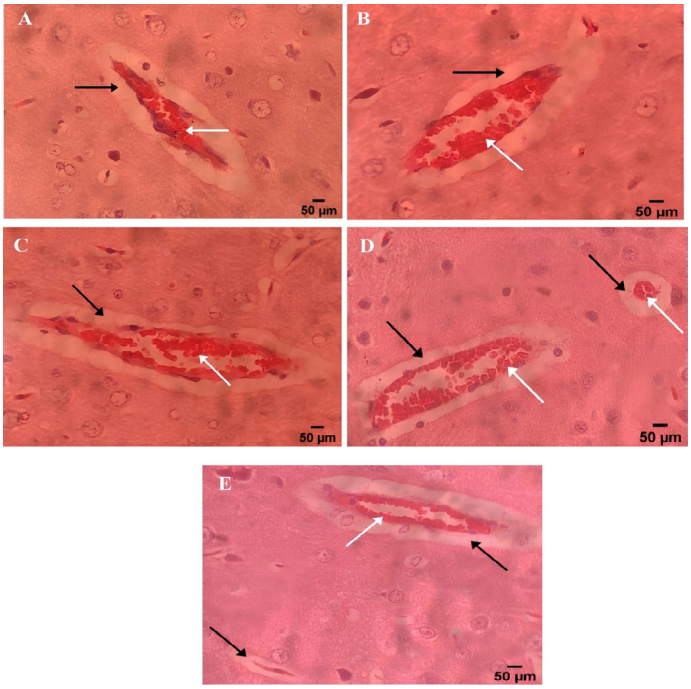

Figure 1: Histopathological images of the brains of white rats (*Rattus norvegicus*). **A:** Ketamine, 80 mg/kg; **B:** 100 mg/kg; **C:** 300 mg/kg; **D:** 500 mg/kg; **E:** 700 mg/kg. Congestive lesions (white arrows) and perivascular edema (black arrows) are evident (HE, 400x).

This CNS suppression plays a vital role in inducing sedation or light anesthesia, making saponins relevant to the observed pharmacological effects. Tannins, while often associated with astringent properties, also contribute to anesthetic action through a different mechanism. Tannins can inhibit activity directly, limiting the degree of hydrolysis, and indirectly decreasing the H2 availability (Besharati *et al.*, 2022). By lowering metabolic activity, tannins help reduce the rate of physiological processes, which may limit waste products' excretion and help maintain homeostasis during anesthetic exposure (Khan, 2019). This metabolic stabilization can be particularly beneficial in prolonging the anesthetic state and minimizing physiological stress.

Links Researchers

The combined presence of these three classes of secondary metabolites, each acting through distinct but complementary mechanisms, provides a biochemical basis for the anesthetic effects observed in the administration of *Datura metel L*. flower extract.

The OECD 425 toxicity test showed that reflex to the stimulus with ears in mice, hypotonia observed at 550 mg/kg and 2000 mg/kg is characterized by reduced motor activity and limb weakness. Regardless, no change in behaviour or death was noted during the fourteen-days observation period, indicating high tolerance for these doses of the extract. Using AOT425 StatPgm (Acute Oral Toxicity (Guideline 425) Statistical Program) software to estimate, the LD50 value was greater than 2000 mg/kg, which places the extract as non-toxic based on BPOM RI (Badan Pengawas Obat dan Makanan Republik Indonesia, 2014), where substances with LD50 > 2000 mg/kg are regarded as non-toxic and classified as such under acute toxicity.

The anesthetic effects of *Datura* are aided by the presence of saponins, tannins, and alkaloids. Alkaloids, including hyoscyamine, hyoscine, and atropine, are known to affect the central nervous system and its components, like the brain and spinal cord, as well as the autonomic nervous system (Sayhan et al., 2017; Igben *et al*., 2023). These compounds anticholinergically inhibit acetylcholine action at muscarinic (mAChR) and partially at nicotinic (nAChR) receptors (Shim *et al*., 2022). Tropane alkaloids also influence sodium channels, which are essential for generating and propagating action potentials. These alkaloids can modulate the activity of voltage-gated sodium channels, contributing to their antinociceptive and muscle relaxant properties (Shim *et al*., 2022). Saponins additionally disrupt the function of the central nervous system by disturbing the balance of cations within the brain, leading to hemolytic conditions and a decline in oxygen supply available for cellular energy (Abid *et al*., 2014; Harfiani *et al*., 2020). In contrast, tannins curb excessive metabolic activity, which assists in the maintenance of homeostasis (Ilhami et al., 2015).

The lack of sedation noted in the current study is likely due to the extracts' systemic route. At this level, the effects may be limited to the cardiovascular system, resulting in bradycardia (Maheshwari et al., 2013; Sudisma *et al*., 2023), which was not measured by the evaluated parameters. Alkaloids are known to have effects on the central nervous system. However, their effectiveness highly depends on their ability to penetrate the blood-brain barrier (BBB) and the dose used. First-pass metabolism in the liver can reduce the concentration of the active compound reaching the CNS, so sedative effects may not occur at low doses or in certain dosage forms (Kohnen and Kayser, 2019). The presence of crude extracts, which are deemed to contain anthraquinones and steroids as either passively active or wholly passive, passive parts that make up the crude extract, increases the claimed potency. The unexpected findings may stem from variability in individual pain responses, treatment in the rats, and differential pharmacokinetic processes within rat populations. Furthermore, passive oral routes may be less effective because extract binding to receptors is not immediate due to sequestration through metabolism and absorption.

All hematological parameters observed following administration of *Datura metel* extract remained within normal physiological limits, despite showing dose-related variations. These findings suggest that the extract influences blood composition. The presence of alkaloids in *Datura* is a likely contributor to these changes, as known act as competitive antagonists of muscarinic cholinergic receptors, thereby affecting autonomic regulation and potentially interfering with the production and function of blood cells (Lakstygal *et al*., 2019; Shim *et al*., 2022). Increasing the dosage of *Datura stramonium* extract has been associated with elevated haemoglobin concentrations and stimulation of erythropoiesis. This is supported by previous findings from Njoya *et al*. (2024), who reported increased mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV), indicating an increase in erythrocyte volume and hemoglobin content per cell. Variations in total erythrocyte counts across different doses may reflect opposing mechanisms: either an erythropoietin-mediated stimulation of red blood cell production or extract-induced erythrocyte lysis and degradation, as suggested by Imo *et al*. (2016). The increased hematocrit at the highest dose (700 mg/kg) exceeded the upper normal limit of 46%, potentially leading to increased blood viscosity and enhanced tissue perfusion. In contrast, leukocyte counts showed a consistent dose-dependent decline, which may reflect a suppressive effect on the immune system. This could result either from disruption of hematopoietic processes or from immune modulation by the alkaloid components of the extract (Imo *et al*., 2016; Olusola *et al*., 2023).

This study has several limitations. It lacked molecular studies (e.g., HPLC or LC-MS analysis), the phytochemical analysis was only qualitative and active compounds were not quantified. Histopathological evaluations of other vital organs were conducted to confirm the dose-response relationships and the mechanisms of anesthetic effects. Additionally, the 14-days observation period did not permit long-term toxicity evaluation. Hematology interpretation is limited to hemoconcentration indicators; further research is recommended to evaluate the long-term hematological impact and fluid balance effects at higher doses. Importantly, since the extract was administered orally, alternative routes (e.g., intraperitoneal or intravenous) may yield different pharmacological or toxicological outcomes, which were not explored in this study. It is important to

note that this research represents a preliminary investigation, primarily focused on evaluating the potential effects of *Datura metel* flower extract via the oral route. Future studies are encouraged to build on these findings by exploring different administration methods, optimizing formulation strategies, and conducting more comprehensive dose-response assessments.

CONCLUSIONS AND RECOMMENDATIONS

Considering the results of this investigation, the 95% methanol extract of *Datura metel L*. var. *arebis* flowers was found to contain alkaloids, saponins, and tannins, supporting its potential as a natural anesthetic agent. Probit analysis of acute oral toxicity data indicated an LD_{50} value above 2000 mg/kg, classifying the extract as non-toxic according to OECD guidelines. The extract exhibited notable strong analgesic effects with mild relaxation and no observable sedative action. Furthermore, administration of doses at 100, 300, and 500 mg/kg did not significantly alter hematological parameters including hemoglobin, hematocrit, erythrocyte, leukocyte, and monocyte counts, which remained within physiological reference ranges. Histopathological evaluation of brain tissue also revealed no significant morphological alterations, further supporting the extract's safety profile as an anesthetic candidate. These findings suggest that *Datura metel* extract, within the tested dose range, possesses anesthetic potential with minimal toxicity risk under oral administration.

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NOVELTY STATEMENT

This study provides new insights into evaluate the potential effect of datura flower (*Datura metel L.*) extract as an anesthetic based on phytochemical test results, toxicity, anesthesia triad, hematology, and histopathology.

AUTHOR'S CONTRIBUTIONS

All authors did the manuscript together and the contribution is distributed equally.

Conflict of Interest

The authors have declared no conflict of interest.

REFERENCE

Abid MS, Masithah ED, Prayogo (2014). Potensi senyawa metabolit sekunder infusum daun durian (*Durio zibethinus*) terhadap kelulushidupan ikan nila (*Oreochromis niloticus*) pada transportasi ikan hidup sistem kering. J. Ilm. Perikan. Kelautan, 6(1):93-99. https://doi.org/10.20473/jipk.v6i1.11386

Al-Huqail AA, Elgaaly GA, Ibrahim MM (2018). Identification of Bioactive Phytochemical from Two Punica Species Using GC–MS and Estimation of Antioxidant Activity of Seed Extracts. Saudi J. Biol. Sci., 25(7): 1420–1428. DOI. 10.1016/j.sjbs.2015.11.009

Archana AM, Lokesha MS, and Manjunath H (2023). Qualitative Phytochemical Analysis of *Datura* (*Datura metel* Linn.). Pharm. Innov. J., 12(12): 110–115. https://www.thepharmajournal.com/archives/2023/vol12issue12/PartAW/12-11-110-773.pdf

Armansyah TR, Indriany S, Sutriana A (2016). Uji toksisitas akut ekstrak etanol daun malaka (*Phyllantus emblica*) terhadap mencit (*Mus musculus*). J. Kedokteran Hewan, 10(2).

Badan Pengawas Obat Dan Makanan (BPOM) (2014). Peraturan kepala badan pengawas obat dan makanan Republik Indonesia nomor 2 tahun 2014 tentang organisasi dan tata kerja unit pelaksana teknis di lingkungan BPOM. *BPOM*.

Besharati M, Maggiolino A, Palangi V, Kaya A, Jabbar M, Eseceli H, De Palo P, Lorenzo JM (2022). Tannin in Ruminant Nutrition: Review. Molecules, 27: 8273. https://doi.org/10.3390/ molecules27238273

Carpa R, Dumitru DV, Burtescu RF, Maior MC, Dobrotă C, Olah NK (2017). Biochemical analysis of Datura stramonium extract. Studia Univ Babes. Bolyai Biol., 62(2): 5–19. https://doi.org/10.24193/subbbiol.2017.2.01

Eltayeib AA, Siddige ANTM (2021). Histological and body weight effects of *Datura innoxia* seeds and leaves extracts in rats. Int. J. Front. Life Sci. Res., 1(1):18-27. https://doi.org/10.53294/ijflsr.2021.1.1.0032

Faqihudin MS, Hidayati S, Aji MT (2023). The effect of anesthesia of basil (*Ocimum basilicum*) leaf extract with different doses on the survival of catfish (*Pangasius* sp.). Asian J. Aquat. Sci., 6(1):102-107. https://doi.org/10.31258/ajoas.6.1.102-107

Ganesh S, Radha R, Jayshree N (2015). A review on phytochemical and pharmacological status of *Datura fastuosa* Linn. Int. J. Multidiscip. Res. Dev., 2(4):602-605.

Gente M, Leman MA, Anindita PS (2015). Uji efek analgesia ekstrak daun kecubung (*Datura metel L.*) pada tikus wistar (*Rattus norvegicus*) jantan. e-GiGi, 3(2): 470-475. DOI. https://doi.org/10.35790/eg.3.2.2015.9838

Harfiani E, Pradana DL, Yusmaini H (2020). A Review on the Phytochemical and Pharmacological Activitities of *Luffa acutangula (L.) Roxb*. Pharm. J. Indones., 12(2): 396-406. https://doi.org/10.30595/pharmacy.v17i2.8220

Hasen G, Hashim R (2021). Current Awareness of Health Professionals on the Safety of Herbal Medicine and Associated Factors in the South West of Ethiopia. J. Multi. Healthcare, 12: 2001-2008. https://doi.org/10.2147/JMDH.S321765

Igben VO, Iju WJ, Itivere OA, Oyem JC, Akpulu PS, Aham EE (2023). *Datura metel stramonium* exacerbates behavioral deicits, medial prefrontal cortex, and hippocampal neurotoxicity in mice via redox imbalance. Lab. Anim. Res., 39(15): 1-19. https://doi.org/10.1186/s42826-023-00162-7

Ilhami R, Ali M, Putri B (2015). Transportasi Basah Benih Nila

(Oreochromis niloticus) menggunakan Ekstrak Bunga Kamboja (*Plumeria acuminata*). J. Rekayasa dan Teknologi Budidaya Perairan, 3(2): 389-396.

Imo C, Arowora KA, Awache I, Abdullahi ZR (2016). Haematological effects of ethanolic leaf, seed supported by and fruit extracts of datura metel on male albino rats. FUW Trends Sci. Technol. J., 1(2): 509 – 512.

Khan W, Subhan S, Shams DF, Afridi SG, Ullah R, Shahat AA, Alqahtani AS (2019). Antioxidant Potential, Phytochemicals Composition, and Metal Contents of Datura alba. Hindawi BioMed. Res. Int., 2019(2403718): 1-8. https://doi.org/10.1155/2019/2403718

Kohnen JKL, Kayser O (2019). Tropane Alkaloids: Chemistry, Pharmacology, Biosynthesis and Production. Molecules, (Basel, Switzerland), 24(4): 796. https://doi.org/10.3390/molecules24040796

Lakstygal A, Kolesnikova T, Khatsko S, Zabegalov K, Volgin A, Demin K (2019). DARK classics in chemical neuroscience: Atropine, scopolamine, and other anticholinergic deliriant hallucinogens. *ACS* Chem. Neurosci., 10:2144-2159. https://doi.org/10.1021/acschemneuro.8b00615

Madziga HA, Sanni S, Sandabe UK (2010). Phytochemical and Elemental Analysis of Acalypha wilkesiana Leaf. J. Am. Sci., 6(11): 510-514.

Mbaye M, Sall C, Djigal D, Faye O, Sylla SM, Ndong A, Ndao M, Sarr D, Coly NF, Soumboundou M (2025). Evaluation of *Datura metel* Leaves Extracts for Their Antioxidant and Nematicidal Properties Against *Meloidogyne javanica* as a Potential Biopesticide. Chem. Sci. Int. J., 34(2): 47–56. https://doi.org/10.9734/CSJI/2025/v34i2956

Maheswari N, Khan A, Chopade BA (2013). Rediscovering the medicinal properties of Datura sp.: A review. J. Med. Plants Res., 7(39): 2885-2897. DOI. https://doi.org/10.5897/JMPR11.1657

Naor L, Mayseless O (2020). The art of working with nature in nature based therapies. J. Exp. Educ., 40: 72–86.

Njoya HK, Christina OU, Vania ED (2024). Effects of *Datura stramonium* crude ethanolic leaf extract on some hematological indices in adult male Wistar albino rats. Int. J. Biosci. Biochem., 6(1):29-37. https://doi.org/10.33545/26646536.2024.v6.i1a.63

Olusola SE, Ayebidun OV, Amubieya AV, Lamulejoye FD (2023). Impact of different concentration of thorn apple (*Datura stramonium*) extract on growth, histopathology, and blood profile response to sub-lethal exposure of Nile tilapia (*Oreochromis niloticus*) juveniles. J. Appl. Sci. Environ. Manage., 27(1):183-190. https://doi.org/10.4314/jasem.v27i1.26

Pemayun IGAGP, Sudisma IGN (2018). Anestesi tetes infus gravimetrik ketapol sebagai alternatif bius umum secara inhalasi guna menjaga status teranestesi pada babi. J. Vet., 19(1):126-136. https://doi.org/10.19087/jveteriner.2018.19.1.126

Pratiwi IN, Aligita W, Kaniawati M (2021). Kajian potensi antioksidan dari tanaman herbal dan pengaruhnya terhadap penyakit Parkinson. J. Ilm. Farm., 17(1):80-95. https://doi.org/10.20885/jif.vol17.iss1.art9

Prihatiningsih NLP, Sudisma IGN, dan Pemayun IGAGP (2022). Respon Analgesia, Sedasi, dan Relaksasi Anestesi Ketamin dan Propofol dengan Premedikasi Xilasin pada Sapi Bali. Buletin Vet. Udayana, 14(5): 511-516. https://doi.org/10.24843/bulvet.2022.v14.i05.p10

Rokhmah NN, Yulianita, Suherlan MFJ (2021). Efek sari buah cherry (*Prunus avium* (L.)) terhadap efek sedasi dan waktu tidur pada mencit putih jantan. Pharmacy, 18(1). https://doi.org/10.30595/pharmacy.v18i1.9233

Samuel J, Sudisma IGN, Dada IKA (2018). Respon analgesia, sedasi dan relaksasi tikus putih yang diberi ekstrak biji kecubung (*Datura metel* L.) intraperitoneal. Indones. Med. Veterinus, 7(1):16-24. https://doi.org/10.19087/imv.2018.7.1.16

Saputra A, Putri RMS, Apriandi A (2021). Grouper Imotilization Technique Epinephelus sp. Using *Datura Metel L.* Seed Extract. J. Mar. Coastal Sci., 10 (3): 105-117. https://doi.org/10.20473/jmcs.v10i3.27234

Savitri ES, Holil K, Resmisari RS (2022). Phytochemistry Screening and Antioxidant Activities of Extract Pomegranate, Grape, Fig, and Olive in the Various Solvent. Jurnal Biodjati, 7(1): 132–139.

Sayhan H, Beyaz SG, Çeliktaş A (2017). Chapter 3. The local anesthetic and pain relief activity of alkaloids. Alkaloids - Altern. Synth. Modif. Appl., pp. 75. DOI. https://doi.org/10.5772/intechopen.69847

Shim KH, Kang MJ, Sharma N, An SSA (2022). A review - Beauty of the beast: Anticholinergic tropane alkaloids in therapeutics. Nat. Prod. Bioprospect., 12(33):2-15. https://doi.org/10.1007/s13659-022-00357-w

Sholichah, Sudisma IGN, Wardhita AAGJ (2017). Efek trias anestesi ekstrak daun kecubung (datura metel l.) pada tikus putih (rattus norvegicus). Indones. Med. Vet., 6(5): 399-408.

Sudira IW, Samsuri, Sudisma IGN, Diana KLM (2024). Phytochemical Tests of 70% Ethanol Extract and Amethyst Water Extract (*Datura metel L.*) which has Potential as Anesthetics. J. Sain Veteriner, 42(3): 380-388. https://doi.org/10.22146/jsv.74161

Sudisma IGN, Soma IG, Sudira IW, Rastiti NM (2023). Clinical and Physiological Response of White Rats (Rattus Norvegicus) Given Amethyst Flower Extract (*Datura Metel L.*) as Anesthesia. J. Sain Veteriner, 41(3): 323-335. https://doi.org/10.22146/jsv.74314

Sukariada IPJ, Sudira IW, Sudisma IGN (2016). The effectivity of ethanol extract of *Datura metel* L. seeds as a general anesthesia on Kintamani dogs. Vet. Sci. Med. J., 4(1):27-31.

Suryadi AMA, Mustapa MA, Hiola F, Basiru S (2021). Uji Aktivitas Antibakteri Ekstrak Metanol Daun Kecubung (*Datura metel L.*) Terhadap Bakteri Streptococcus pneumonia Dan Klabsiella. Indones. J. Pharm., (e-Journal),1(3): 179-189.

Tamalawe AK, Potalangi NO, Tulandi HV, Paat VI (2021). Uji Potensi Efek Sedatif Ekstrak Bunga Kecubung *Datura metel Linn* Pada Tikus Putih Jantan *Rattus norvegicus*. Majalah Inf. Sains, 2(1): 38-47. https://doi.org/10.55724/jis.v2i1.21

Variani YA, Setyaningrum E, Handayani K, Nukmal, Arifiyanto A (2021). Analisis senyawa bioaktif ekstrak metabolit sekunder *Serratia marcescens* strain MBC1. Indones. J. Chem. Anal., 4(2):64-71. https://doi.org/10.20885/ijca.vol4.iss2.art3

Waqas A, Haroon K, Sajjad AK, Sana N, and Esra KA (2021). *Datura metel*: A Review on Chemical Constituents, Traditional Uses and Pharmacological Activities. Curr. Pharm. Des., 27(22): 2545-2557. https://dx.doi.org/10.2174/1381612826666200519113752