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Short communication

Sedative-hypnotic effects of *Datura arborea* Linn extract in experimental animals

Aisha Idris [1 *] and **Shamsiya Idris** [2]

[1] Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, and [2] Department of Biology, Faculty of Life Sciences, College of Science, Kaduna State University, Kaduna, Nigeria
[*]Author to whom correspondence should be addressed

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Abstract: *Datura arborea* Linn is a sacred plant known for over 3000 years to have been used for magical and curative purposes. It was shown to have a central nervous system depressant effect. The active substances identified were tropane alkaloids: atropine, scopolamine, and hyosine. Therefore, we aimed to find out whether the ethanol extract of *Datura arborea* Linn has sedative and hypnotic activity. The extract was subjected to a thiopental sodium-induced sleep test and diazepam was used as a standard drug. The plant possesses sedative-hypnotic qualities, the findings indicate that doses of 35 mg/kg (2.70.24 min), 70 mg/kg (3.80.19 min), and 140 mg/kg (4.30.20 min) decreased the control's (9.2 min) latency to fall asleep. In comparison to the control, the length of sleep was increased by 23.46 minutes for 35 mg/kg (99.002.99 min), 70 mg/kg (132.605.53 min), and 140 mg/kg (118.606.04 min), respectively. The present study established the acute toxicity of *Datura arborea* Linn to be less than 600 mg/kg in mice. This study indicated that the ethanol extract of *Datura arborea* Linn has a sedative-hypnotic activity in the diazepam-induced sleep test. It is safe to suggest that the extract acts via either β-receptor by causing hyperpolarization or a decrease in spike activity in the cell, leading to relaxation, but these effects were not blocked by β-antagonist or α_2-receptor to decrease acetylcholine release, leading to relaxation of the smooth muscle.

Introduction

Healthy sleep is important for cognitive functioning, mood, mental health and cerebrovascular, cardio-vascular, and metabolic health [1]. Short-term sleep deprivation, long-term sleep restriction, circadian misalignment, and untreated sleep disorders can have a profound and detrimental impact on physical health, mental health, mood, and public safety [2, 3]. Chronic insufficient sleep has been associated with an increased risk of mortality and contributes to the individual risk and societal burden associated with several medical epidemics, including cardiovascular disease, depression, anxiety, diabetes, obesity, and cancer [4-8]. Emergent data suggest qualitative sleep has its perks and is associated with health benefits such as the reduced risk of obesity, type 2 diabetes, and cardio-metabolic health, among others [1, 9-12]. *Brugmansia Pers, Datura L., and Iochroma Benth* have been considered to form the *Datureae* Tribe of the family Solanaceae [13]. The plants of this genus are large perennial shrubs or small trees, usually

distributed in the world as ornamental plants, especially in tropical and subtropical to temperate regions [4, 14]. Species of this genus since ancient times were used as hallucinogenic drugs and medicines [14]. *Brugmansia* species have been used as folk medicines in North and South America to treat headaches, rheumatic arthritis, inflammations, skin infections and other diseases [15, 16]. The species of the genus *Brugmansia* are a rich source of tropane alkaloids chiefly atropine and scopolamine, which has interesting therapeutic effects, including antiaddictive, antispasmodic, antiasthmatic, narcotic, and antinociceptive activity [17-20]. In recent years, several studies have shown that the flavonoids, monoterpenes, and benzonitrile glycosides isolated from this genus possess significant pharmacological activities, including cytotoxicity, immunomodulatory, antioxidant, anti-inflammatory, and so on [14, 21, 22]. We sought to determine whether *Datura arborea* Linn's (**Figure 1**) ethanol extract possesses any sedative-hypnotic activity due to the plant's vast range of defined pharmacological, phytochemicals and the deficiency of studies that have evaluated its sedative-hypnotic effects to date.

Figure 1: *Datura Arborea Linn*

Materials and methods

Experimental animals and materials: Male and female Albino Swiss Wister mice (n=24) with a body weight of 24-30 g, male Albino Swiss Wister rats (n=13) with a body weight of 93-119 g and one male adult New Zealand male rabbit 0.87 kg were used throughout this study.

Datura arborea Linn powdered leaves (135 g) identified by the Department of Biology, Faculty of Life Sciences, College of Science, Kaduna State University, Kaduna, Nigeria was used in this study.

Ethanol plant extraction: Dried powdered leaves of *Datura arborea* Linn weighing 135 g were poured into a separating funnel. The solvent, ethanol, was then added, poured sufficiently to cover the powder, and allowed for 24 hrs, after which it was drained and rewashed with more ethanol. The filtrate was poured into an evaporating dish and placed in a water bath at about 600°C until the water molecules evaporated, leaving the extracted residue. The extracted residue was then placed in a container, and it weighed about 80 g [22].

Acute toxicity studies: The Lorke method was used to determine the LD_{50} in the rat [23]. The study was conducted in two phases using a total of 13 male rats. During the first stage, the plant's ethanol extract was given as intraperitoneally (i.p.) to three groups at dosages of 10, 100, and 1000 mg/kg. The animals were then monitored for signs of toxicity and death for a duration of 24 hrs. In the second stage, four groups with a single rat were given four additional precise dosages of the extract by injection (600, 370, 225, and 140 mg/kg, respectively). The geometric mean of the lowest dose that resulted in death and the greatest dose for which the animal survived (0/1 and 1/1) was used to calculate the LD_{50} value.

All the experiments performed on laboratory animals were by Ahmadu Bello University Research policy as well as ethics and regulations governing the care and use of experimental animals as contained in "Principles of Laboratory Animal Care" published by the National Institute of Health (NIH Publication No. 85-23, revised, 1996).

Diazepam-induced sleep time in mice: The method described early by Beretz et al. [24] and modified by Rakotonirina et al. [25] was used. 24 mice were randomly divided into four groups, each group containing six mice. The first group served as a control and was given only diazepam in a dose of 20 mg/kg, i.p. The second, third, and fourth groups were

given 35, 70, and 140 mg/kg, i.p., respectively. The time between the loss of the straightening reflex and the regain of this reflex measured the sleeping time. The loss or gain of the straightening reflex was measured by stimulating the external ear. When the mouse's anterior paw does not move after stimulation with horsehair, the mouse is sleeping. When the mouse is awake, it moves its paw. The loss of the righting reflex was considered the criterion for sleep [26], while the interval between the loss and the recovery of straightening was taken as the duration of sleep [27].

Rabbit's isolated ileum studies: Modified Magnus technique was used [28, 29]. Briefly, the rabbit was stunned, and the abdomen opened with a pair of scissors. The intestines were gradually removed and sections of the jejunum were cut. Suitable lengths (2-3 cm) were fixed with a tissue clamp and suspended in a 25 mL organ bath containing Tyrode's solution. The solution was oxygenated with air bubbles using an air pump and maintained at 37°C using a thermo-circulator. The lower end of the tissue was attached to an oxygenated tube, while the upper end was fixed to an isometric force transducer. After a pre-incubation time of 30 min, the experiments were started [28, 29].

Statistical analysis: All the values were expressed as mean±SEM. The statistical differences in the mean latency time to sleep and duration of sleep among the groups of mice were tested by one-way ANOVA as an overall difference followed by Scheff's post-hoc test. The linear regression test was used to determine the dose dependency of the observed effects and a p-value ≤ 0.05 was considered to be statistically significant.

Results and discussion

Acute toxicity studies: In **Table 1,** the acute lethal effect studies on rats showed that no animal died within 24 hrs after treatment with the plant ethanolic extract. The major signs of toxicity noticed within 24 hrs included difficulty breathing, loss of appetite, and general weakness. There was 100% death in the 1000 mg/kg body weight dose group. The second phase has shown progress and has become increasingly pronounced as the dose decreased towards 500 mg/kg with death at 600 mg/kg, it is safe to conclude the LD_{50} is less than 600 mg/kg [23].

Table 1: Acute lethal effect of ethanol extract of *Datura arborea Linn* on rats

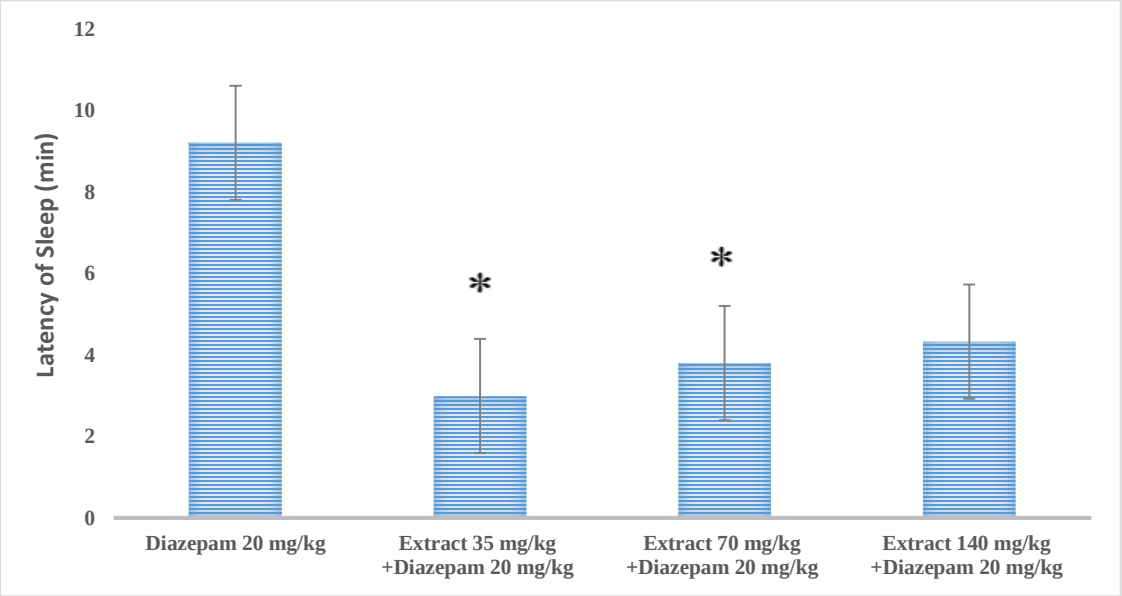
Experiment	Dose (mg/kg)	Dead rats after 24hr	Treated rats after 24 hr
Phase-1*	10	0/3	0/3*
	100	0/3	0/3
	1,000	3/3	3/3
Phase 2	140	0/1	0/1
	225	0/1	0/1
	370	0/1	0/1
	600	1/1	1/1

*Experiment conducted in 2 phases; each dose group of phase-1 made up of 3 rats while those in phase 2 have 1 rat per group.

Rabbit isolated ileum studies: The rabbit tissue experiment of the extract result showed a relaxation effect (decreased frequency and amplitude of contraction) which is similar to adrenaline. It is safe to suggest that the extract acts via either β-receptor by causing hyperpolarization or a decrease in spike activity in the cell leading to relaxation, but, the observed effect was not blocked by β-antagonist (propranolol), α_2-receptor to decrease acetylcholine release leading to relaxation of the smooth muscle.

*Latency of sleep***:** Etanolic extract *Datura arborea* Linn strongly potentiated in a dose-dependent manner the latency of sleeping time induced by diazepam from 9.2±2.25 min in the control group compared to the treated groups with diazepam 35 mg/kg, 70 mg/kg and 140 mg/kg at 2.796±0.24, 3.808±0.19 and 4.33±0.20, respectively (**Figure 2**). A comparison between groups 1 and 2 is 0.022 proved to be significant, so, the comparison between groups 1 and 3 is 0.044 but the comparison between groups 1 and 4 is 0.063; but, not significant. Comparison between groups 2 and 3 is 0.011 and between groups 2 and 4 0.001 showing significance unlike the comparison between groups 3 and 4 is 0.088 revealed no significance. This indicates that the extract might have possessed hypnotic activity at lower doses.

Figure 2: Effect of *Datura arborea* Linn on latency of sleep in mice induced by diazepam

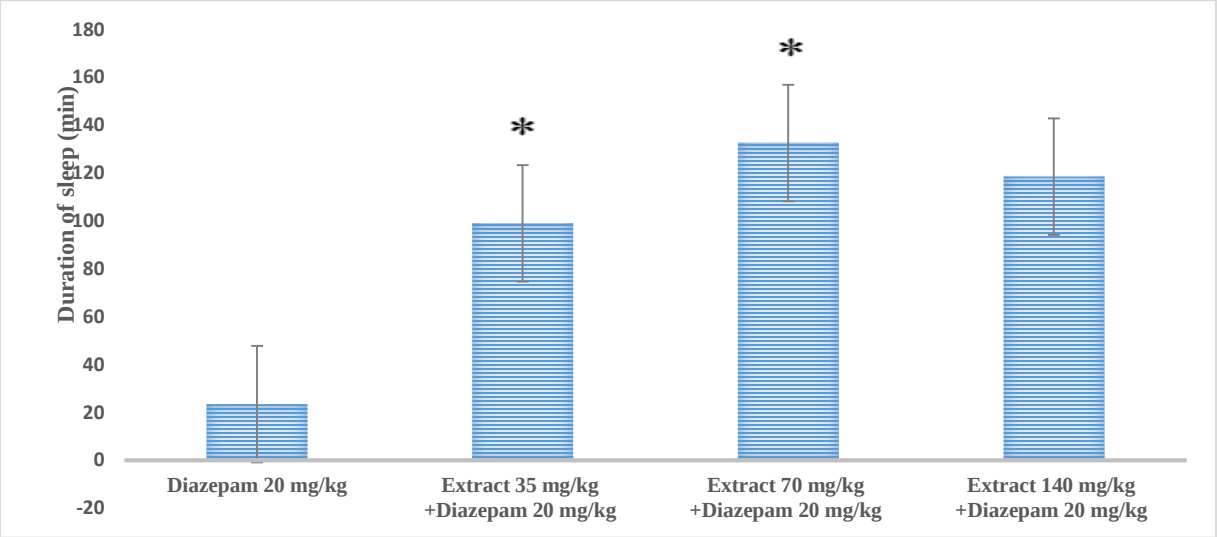


Bars are mean±SEM of the onset time of sleep of mice induced by diazepam in the presence of the extract.
The mean value of CON was compared to the mean values of the groups treated with the extract. n = 5 per dose, * $p < 0.05$.

Duration of sleep: Datura arborea Linn alcoholic extract strongly potentiated in a dose-dependent manner the duration of sleeping time induced by diazepam from 23.46±2.99 min in the control group compared to the treated groups with diazepam in a dose of 35 mg/kg, of 70 mg/kg, and of 140 mg/kg at 99.00±2.99 and 132.60±5.53 as well as 118.60±6.04, respectively, (**Figure 3**). The comparison between groups 1 and 2 is 0.008 which is significant, so the comparison between groups 1 and 3 is 0.0012 as well as comparison between groups 1 and 4 is 0.006 considered to be statistically significant. The comparison between groups 2 and 3 is 0.017 showed to be significant. The comparison between groups 2 and 4 is 0.127 and the comparison between groups 3 and 4 is 0.126 but showed statistically not significant. The prolongation in the duration of sleep by the extract might be due to the involvement of the GABA-ergic system.

Regarding the loss of righting reflex [30], a significant decrease in the onset of sleep due to loss of locomotor activity was observed in the alcoholic extract of *Datura arborea* Linn that suggests the extract is endowed with central nervous system depressant activity [31] similar to the other genus of Datura [32] as it was reported to have anticholinergic activity [14]. In addition, it has been shown that flavonoids are in this plant can modulate the activity of major inhibitory amino acid neurotransmitter γ-aminobutyric acid (GABA) receptors of subtype-A [33]. Therefore, it's worth suggesting that flavonoids detected in the plant extract may be responsible for the pharmacological activity found in the present study. The sedative effects might suggest that *Datura arborea* Linn acts by interacting with the $GABA_A$ receptor via benzodiazepines binding sites. GABA system is known to play an important role in sleep, and the positive allosteric modulators of $GABA_A$ receptor (e.g., benzodiazepines) are widely used to promote restful sleep [34]. However, further research on the binding assay, the binding affinity to the $GABA_A$-BZD and 5-HT_{2C} receptors can be carried out to assess the definite anxiolytic effects of *Datura arborea* Linn and psychopharmacology studies on depression using animal models could be employed to establish facts on the anti-depression effect.

Figure 3: Effect of *Datura arborea* Linn on the duration of sleep in mice induced by diazepam



Bars are mean±SEM of the duration time of sleep (min) of mice induced by diazepam in the presence of the extract. The mean value of control was compared to the mean values of the groups treated with the extract. n = 5 per dose, * $p < 0.05$.

Conclusion: This study confirms the acute toxicity of *Datura arborea* Linn to be less than 600 mg/kg in experimental animals. The ethanol extract of *Datura arborea* Linn has a sedative-hypnotic activity in the diazepam-induced sleep test. In addition, it is safe to suggest that the extract acts via either β-receptor by causing hyperpolarization or a decrease in the spike activity in the cell leading to relaxation, but this effect was not blocked but β-antagonist, α_2-receptor to decrease acetylcholine release leading to the relaxation of the smooth muscle.

Author contribution: AI conceived, designed the study, analyzed and collected the data. SI had interpreted the data and drafted the manuscript. Both authors approved the final version for publication.
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Ethical issues: Including plagiarism, informed consent, data fabrication or falsification, and double publication or submission have completely been observed by authors.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.

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