ORIGINAL ARTICLE

Anti-seizure effects of Datura stramonium: Impact on neurobehavior, antioxidant enzymes, and inflammatory markers in the hippocampus and amygdala of adult female Wistar rats using an isoniazid-induced epileptic model

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Abstract

Background: Epilepsy, marked by recurrent seizures and neurochemical imbalances, remains a treatment challenge, particularly in resource-limited settings. *Datura Stramonium* (DS), a plant rich in tropane alkaloids like atropine, scopolamine, and hyoscyamine, has demonstrated potential pharmacological effects on the nervous system. However, its antiepileptic and neuroprotective properties are not well-studied. *Aim and Objectives:* This study assessed the anti-seizure potential of DS and its effects on neurobehavioral parameters, antioxidant enzyme activity, and inflammatory markers in an isoniazid-induced epileptic model in adult female wistar rats. *Material and Methods: DS* extract was prepared using a standard extraction protocol. Group 1 was fed with water and standard feed only; group 2 was induced with 300 mg/kg of isoniazid only; group 3 was given low dose *DS* extract (60 mg/kg) only; group 4 was given high dose *DS* extract (120 mg/kg) only; group 5 (standard drug) was pre-treated with carbamazepine (200 mg/kg) and seizure was induced with isoniazid (300 mg/kg); groups 6 and 7 were pre-treated with low (60 mg/kg) and high (120 mg/kg) dose DS extract respectively, and induced with isoniazid (300 mg/kg).Seizure traits were monitored while the threshold and latency were scored for anti-epileptic assessment. Oxidative stress levels were assessed using the superoxide dismutase, catalase, and malondialdehyde biomarkers, and TNF-α levels were also assessed for pro-inflammatory reactions. The histology of the hippocampus and amygdala, stained with haematoxylin and eosin, was studied. *Results:* The results revealed that low-dose DS (60 mg/kg) significantly reduced seizure latency, increased the seizure threshold, showed mild anxiolytic and improved cognitive abilities in behaviour, and also exhibited neuroprotective effects on the hippocampal and amygdala cytoarchitecture against isoniazid-induced epilepsy. DS

extract also showed significant anti-inflammatory and antioxidant abilities. *Conclusion:* These findings suggest that DS possesses neuroprotective, anti-oxidative, and anti-inflammatory effects, as well as being a potent anti-epileptic agent.

Keywords: *Datura stramonium*, Neuroprotection, Isoniazid-induced Epilepsy, Anti-seizure, Oxidative stress, Plant-based neurotherapy, Neurotoxicity. Carbamazepine, Hippocampus, Amygdala

Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent seizures, often associated with cognitive and psychological impairments [1]. It affects individuals across all age groups, with a slightly higher prevalence in males and the elderly due to age-related brain conditions [2]. Seizures may arise from various CNS insults-structural, metabolic, or toxic-and can lead to progressive neurological damage if unmanaged [3].

While Antiepileptic Drugs (AEDs) remain the cornerstone of treatment, challenges such as drug resistance, adverse effects, and individual variability in response persist [4]. These limitations have led to growing interest in complementary and alternative therapies, especially in resource-limited settings where epilepsy burden is high. Medicinal plants, such as *Datura stramonium* (DS), have shown promise due to their bioactive compounds, including scopolamine, with reported antispasmodic and analgesic properties [5]. However, evidence on DS's anti-seizure efficacy, particularly regarding its effects on behaviour, antioxidant defence, inflammation, and brain structure remains limited.

This study investigated the potential anti-seizure-like effects of DS in an isoniazid-induced seizure model in adult female Wistar rats, focusing on neurobehavioral, antioxidant, anti-inflammatory, and histopathological outcomes.

Material and Methods

Collection, authentication, and preparation of plant material

The plant material was harvested in the wild with the help of herbalist at Eke market, Afikpo North Local Government, Ebonyi State, Nigeria, and was authenticated at the Herbarium unit of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria. DS leaves were air-dried at room temperature for seven days, and the leaves were then pulverized using a mortar and pestle. The ground leaves were then further pulverized using an electric blender. The coarse powder was sieved to obtain finer particles. The leaf was extracted following standard extraction procedures [6]. Isoniazid (Lannett, USA) and carbamazepine (Acetol) were procured from a registered pharmaceutical company in Enugu State, Nigeria.

Animal care and management

All protocols and treatment procedures adhered strictly to the guidelines set forth by the Animal Care and Use Committee. Adult female Wistar rats used in this study were procured from the animal house of Department of Veterinary Anatomy, University of Nigeria, Nsukka. The experimental animals were housed in clean individual cages with unrestricted access to food and water, ensuring their well-being throughout the experiment.

Experimental design

We choose to use female Wistar rats for this study based on the consideration that female rats tend to exhibit more consistent baseline behavioural responses and lower levels of aggression compared to males, which reduces variability and stress-related confounding factors in experimental models, particularly in studies involving repeated behavioural assessments [7]. Thirty-five (35) female Wistar rats weighing between 156 and 160g were randomly assigned to seven different groups, five (5) per group. Doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg of DS extract have been evaluated in pharmacological studies [8]. The 60 mg/kg and 120 mg/kg dose of DS used falls within effective dose range from pharmacological studies [8]. The 300 mg/kg of isoniazid used to induce seizures in female albino Wistar rats was well-established for seizure models [9].

DS was administered orally using a cannula and insulin syringe for 21 consecutive days, while the isoniazid induction was given for four consecutive days (from 22nd to 25th days). The experiment lasted for 25 days. From the 22nd day, after administering isoniazid, the epilepsy onset threshold was measured using a stopwatch, recording the interval from administration to the start of convulsion, and the latency was also recorded, all in seconds. Behavioural tests were performed before and on the last day of the experiment.

The Y-maze test was performed, and the animals were brought to the testing room two hours before the commencement of the test. In the trial stage, one arm of the Y-maze apparatus was blocked, and each animal was introduced to explore for five minutes. After an hour, the testing stage commenced; the previously blocked arm was opened, and each animal was introduced into the maze again and allowed to roam for another five minutes. An arm entry scores when all four animal limbs have crossed the centre line into the arm of the maze. Before testing a new animal the apparatus was cleaned with 5% ethanol to eliminate possible bias due to odours left by the previous animal [10-11].

Table 1: Animal groups treated with various dose of drug and treatment agent

Group	Number of Animals	Dose of treatment agent	Dose of Isoniazid
1 (Normal control)	5	Nil	Nil
2 (Positive control)	5	Nil	300 mg/kg
3 (Low-dose negative control)	5	60 mg/kg (DS)	Nil
4 (High-dose negative control)	5	120 mg/kg (DS)	Nil
5 (Standard drug group)	5	200 mg/kg (Carbamazepine)	300 mg/kg
6 (Low-dose treatment)	5	60 mg/kg (DS)	300 mg/kg
7 (High-dose treatment)	5	120 mg/kg (DS)	300 mg/kg

DS = Datura stramonium extract

Each animal was placed in the centre of the elevated plus maze facing the open arm and allowed to explore for 5 minutes [12]. This was recorded using a video-tracking.

Neurobehavioral parameters assessed include mean threshold scores measured in seconds (the period between removing the syringe from the animals and the onset of seizures), mean latency score measured in seconds (the period between the onset of seizures and their termination), mean number of open and closed-arm entries made by the animals across all the groups (arm entry I is the mean number of arm entries made from the tests carried out before the commencement of administration while arm entry II is the mean number of arm entries made by the animals after administration), and mean percentage alternation scores [13-14].

Tumor Necrosis Factor-Alpha (TNF-α)

The sensitivity range of the TNF-α Enzyme-Linked Immunosorbent Assay (ELISA) kit used was 9.38 pg/ml, with a detection range of 15.625-1000 pg/ml. The ELISA procedure was conducted according to the kit manufacturer's protocol.

Superoxide Dismutase (SOD)

SOD activity in the supernatant was measured using a spectrophotometric method. The inhibition of epinephrine autoxidation by SOD was quantified, and the percentage inhibition was calculated.

Catalase (CAT)

CAT activity was determined spectrophotometrically by measuring the breakdown of hydrogen peroxide, following the Beers and Sizer method [14].

Malondialdehyde (MDA)

Lipid peroxidation was determined by measuring the concentration of MDA in the brain tissues using the thiobarbituric acid reactive substances (TBARS) assay [15].

Brain tissue collection and histology

Rats were anesthetized using 0.5 ml of ketamine via subcutaneous injection. Perfusion was performed by injecting normal saline into the heart for 3 minutes, followed by 10% formal saline for tissue fixation. The brain tissue was collected, fixed in 10% formal saline, and processed for histological examination with Haematoxylin and Eosin (H&E) stain to assess cytoarchitecture.

Data analysis

Data were analysed using Statistical Package for the Social Sciences (SPSS) version 23 (IBM, USA). One-way analysis of variance was conducted followed by Tukey HSD post-hoc tests used for multiple comparisons. The value of $p < 0.05$ was considered statistically significant. The results of this study were expressed as Mean ± SEM.

Results

The mean threshold scores (seconds) displayed in table 2 was significantly ($p < 0.05$) reduced in 60 mg/kg and 120 mg/kg DS extract compared to positive control and standard drug groups at day 2 and 4. The mean threshold scores for the positive control group increased on day two but subsequently decreased on days three and four. The groups pre-treated with DS extracts (60 mg/kg and 120 mg/kg) showed a slight increase in seizure thresholds on day two, but declined on subsequent days.

Table 3 shows the mean latency scores (in seconds). The mean latency scores were reduced in 60 mg/kg DS extract compared to control and standard drug groups at day 1 and 2.

Table 2: Mean Threshold scores (seconds)

Group	Day 1	Day 2	Day 3	Day 4
2 (Positive control)	2084.80 ± 494.84	4800.00 ± 368.1	2926.50 ± 561.50	2448.5 ± 340.67
5 (Standard drug)	2801.75 ± 866.77	4817.00 ± 651.4	3071 ± 262	5702.00
6 (Low-dose treatment)	2100.50 ± 55.50	2152.50 ± 304.50	1766.50 ± 288.5	1698.25 ± 610.05
7 (High-dose treatment)	2492.8 ± 318.04	2401.25 ± 237.96	2432.33 ± 264.95	1394 ± 673.99
F stat	0.342	14.465	2.575	4.371
***p* value**	0.795	0.013*	0.167	0.042*

Table 3: Mean latency scores

Group	Day 1	Day 2	Day 3	Day 4
2 (Positive control)	53.2 ± 8.29	75.0 ± 0.05	48.5 ± 11.50	27.75 ± 3.57
5 (Standard drug)	60.75 ± 21.31	104.00 ± 1.23	161 ± 41	43.00 ± 0.45
6 (Low-dose treatment)	39 ± 21	48 ± 8.00	49.5 ± 5.50	166.25 ± 10.97
7 (High-dose treatment)	97.4 ± 51.59	77.25 ± 18.48	103 ± 29.84	97.67 ± 43.80
F stat	0.479	0.722	3.19	0.722
***p* value**	0.703	0.589	0.122	0.566

Table 4 shows that the groups pre-treated with low dose DS extracts (60 mg/kg) had more arm entries, while the high-dose DS exhibited reduced activity in both open and closed arms. The DS extract pre-treated group, particularly the low-dose group, had significantly ($p < 0.05$) higher percentage alternation scores. Group 5 treated with the carbamazepine also showed higher mean percentage alternation scores compared to positive control group.

Table 5 shows the mean TNF-α, SOD, Catalase, and MDA levels. The mean was slightly elevated in the positive control group compared to the group pre-treated with DS extract. Groups 6 and 7 pre-treated with DS extracts (60 mg/kg and 120 mg/kg) had elevated SOD and catalase levels, and a reduced MDA level compared to the positive control group.

Table 4: Mean number of closed and open arm entries and mean percentage alternation scores

Groups	EPM closed arm entry	EPM open arm entry	Percentage alternation
1 (Normal control)	2.25 ± 0.25	1.5 ± 0.50	77.5 ± 2.50
2 (Positive control)	3.25 ± 1.03	3.0 ± 1.00	33 ± 23.45
3 (Low-dose negative control)	1.0 ± 0.00	1.00 ± 0.00	67.5 ± 7.50
4 (High-dose negative control)	3.0 ± 1.00	3.0 ± 1.22	44.43 ± 5.57
5 (Standard drug group)	5.5 ± 3.50	5.0 ± 4.00	80.0 ± 12.45
6 (Low-dose treatment)	5.33 ± 2.03	5 ± 2.31	87.5 ± 12.50
7 (High-dose treatment)	1.0 ± 0.00	1.0 ± 0.00	50.0 ± 17.67
F stat	2.13	1.3	5.677
***p* value**	0.100	0.312	0.042*

Table 5: Mean Tumor Necrosis Factor-Alpha (TNF-α) and mean oxidative stress markers

Groups	TNF-α (pg/ml)	SOD (u/mg protein)	Catalase (u/mg protein)	MDA (mmol/mg protein)
1 (Normal control)	7.03 ± 0.28	15.66 ± 0.57 [b, c]	41.57 ± 1.15 [b, c, d, f, g]	5.69 ± 0.20 [b, c, d, e, f]
2 (Positive control)	8.75 ± 0.20	10.15 ± 0.44 [c, d, e, f, g]	28.08 ±0.78 [c, d, f]	8.98 ± 0.24 [c, d, e, f, g]
3 (Low-dose negative control)	7.99 ± 0.15	12.20 ± 0.26 [e]	32.37± 0.72	7.33 ± 0.10 [e, g]
4 (High-dose negative control)	7.80 ± 0.11	13.91 ± 0.55	32.08 ± 0.99	7.28 ± 0.15 [g]
5 (Standard drug group)	7.56 ± 0.15	14.92 ± 0.42	39.12 ±0.60 [b, c, d, f, g]	6.49 ± 0.22
6 (Low-dose treatment)	7.93 ± 0.22	13.77 ± 0.29	31.98 ± 0.65	7.15 ± 0.06
7 (High-dose treatment)		14.69 ± 0.19	30.90 ± 0.80	6.20 ± 0.10
F stat	6.83	18.24	34.26	33.73
***p* value**	0.001*	0.00*	0.00*	0.00*

Footnote: [b,c,d,e,f &g] denotes significantly different from group 2, 3, 4, 5, 6 and 7 respectively at $p < 0.05$.

Histological findings

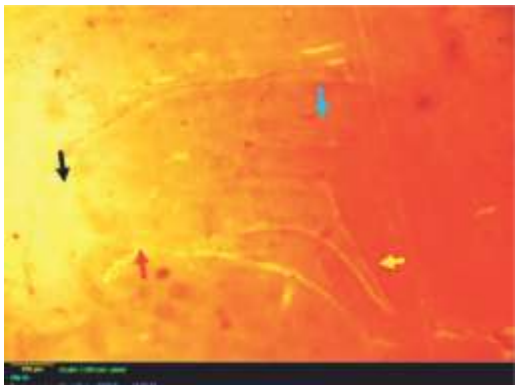

Figure 1: Representative photomicrographs of Haematoxylin and Eosin staining of the hippocampus of Wistar rats in group 1. The dentate gyrus (indicated by the yellow arrow) is composed of both pyramidal and granule cells. Cornus ammonus (CA1-CA3: indicated by blue, black, and red, respectively), containing pyramidal cells, is well demonstrated. The tissue appears normal.

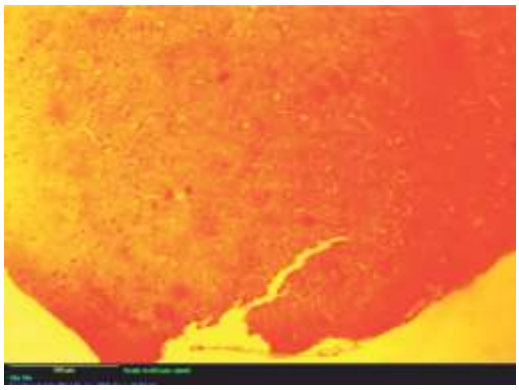

Figure 2: Representative photomicrograph of Haematoxylin and Eosin staining of the amygdala of Wistar rats in group 1. The tissue shows a normal distribution of neuronal cells.

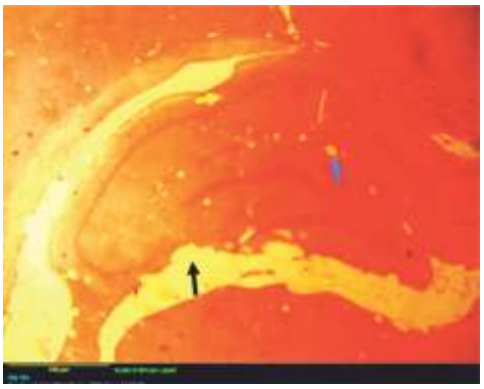

Figure 2: Representative photomicrographs of Haematoxylin and Eosin staining of the hippocampus of Wistar rats in group 2, which were given 300 mg/kg of isoniazid only. Tissue shows sclerosis characterised by depletion, degradation, and neurodegeneration due to an inflammatory response, triggered by injury. There is an end folium pattern of cell loss observed in the hilus, indicated by the black arrow. Perforation within the cell is indicated by blue arrows. Severe dispersion of granule cells into the molecular dentate gyrus. Neuronal loss, predominantly in CA1 and hilar regions, is indicated by the yellow arrow.

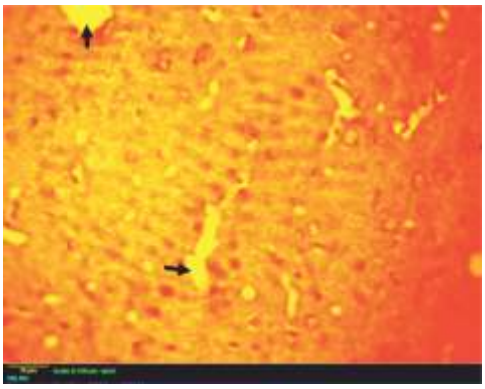

Figure 4: Representative photomicrograph of Haematoxylin and Eosin staining of the amygdala of Wistar rats in study group 2, which were given 300 mg/kg of isoniazid only. Tissue shows great cell loss leading subpopulation of inhibitory neurons, which causes atrophy (reduced volume of organ). Neurodegenerative spotty lesions were seen and are indicated by black arrows.

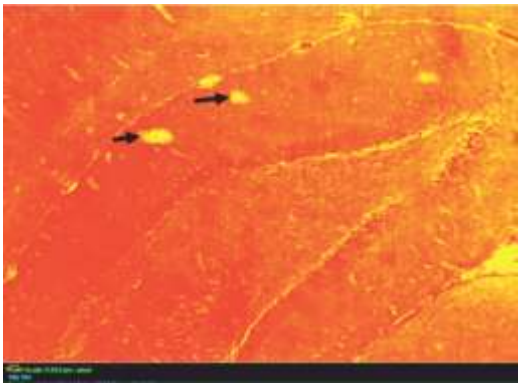

Figure 5: Representative photomicrograph of Haematoxylin and Eosin staining of the hippocampus of Wistar rats in study group 5, which was pretreated with 200 mg/kg of carbamazepine and induced with 300 mg/kg of isoniazid. Tissue shows spotty perinuclear sclerosis (arrows).

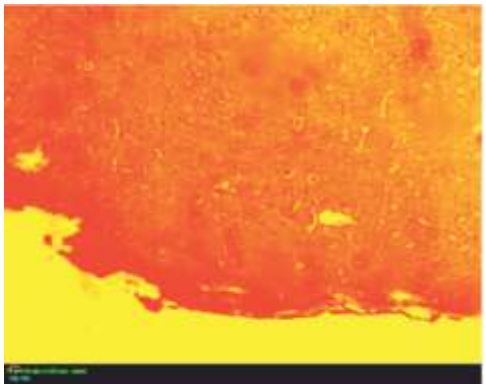

Figure 6: Representative photomicrograph of Haematoxylin and Eosin staining of the amygdala of Wistar rats in study group 5, which was pretreated with 200 mg/kg of carbamazepine and induced with 300 mg/kg of isoniazid. Tissue appears normal.

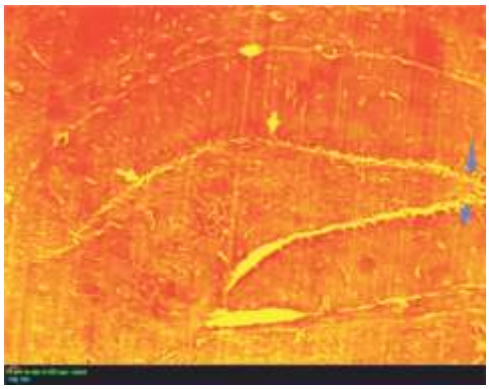

Figure 7: Representative photomicrograph of Haematoxylin and Eosin staining of the hippocampus of Wistar rats in study group 6, which was pretreated with 60 mg/kg of DS and induced with 300 mg/kg of isoniazid. Tissue cytoarchitecture appears normal.

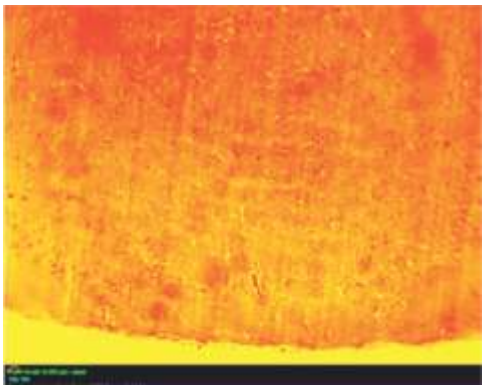

Figure 8: Representative photomicrograph of Haematoxylin and Eosin staining of the amygdala of Wistar rats in study group 6, which was pretreated with 60 mg/kg of DS and induced with 300 mg/kg of isoniazid. Tissue cytoarchitecture appears normal.

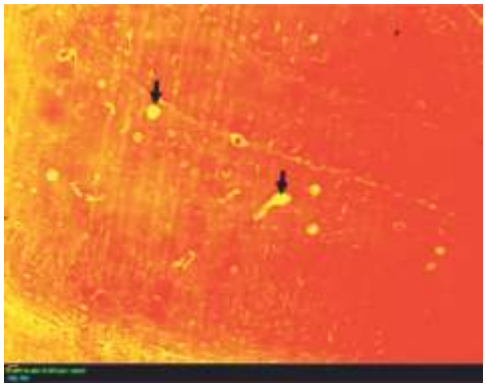

Figure 9: Representative photomicrograph of Haematoxylin and Eosin staining of the amygdala of Wistar rats in study group 7, which was pre-treated with 120 mg/kg of DS and induced with 300 mg/kg of isoniazid. Tissue shows few spotty lesions (arrow).

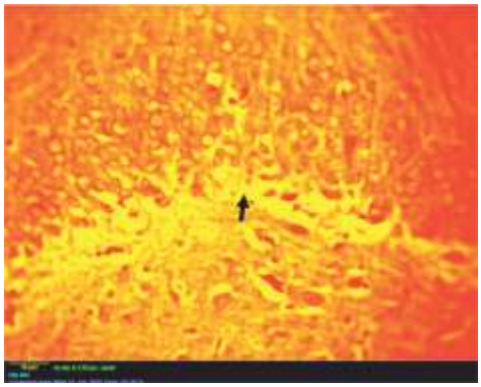

Figure 10: Representative photomicrograph of Haematoxylin and Eosin staining of the amygdala of Wistar rats in study group 7, which was pre-treated with 120 mg/kg of DS and induced with 300 mg/kg of isoniazid. Tissue shows mild neuronal cell loss (arrow).

Discussion

The management of epilepsy remains a significant clinical challenge as a result of the rise in refractory epilepsy cases, which may not respond to standard medications or therapies [16]. Hence, this study investigated the antiepileptic, anxiolytic, and neuroprotective properties of DS leaf extracts as a therapeutic agent against isoniazid-induced epileptogenic-like effects in Wistar rats. The findings of this study will contribute to the growing body of research exploring alternative approaches to managing epilepsy in resource-limited settings.

The results of this study indicate that the mean threshold scores for the positive control group increased on day two but subsequently decreased on days three and four. This decline may reflect the progressive worsening of neuronal hyperactivity as a result of sustained isoniazid exposure. However, the group pre-treated with an antiepileptic drug, carbamazepine (200 mg/kg), exhibited a sustained increase in seizure threshold scores from Days 2 to 4 (Table 2). This finding agrees with the known mechanism of carbamazepine, which stabilizes neuronal membranes and reduces excitability [17]. Interestingly, the groups pre-treated with DS extracts (60 mg/kg and 120 mg/kg) showed a slight increase in seizure thresholds on day two, but declined on subsequent days. This suggests a transient protective effect of DS at both doses, although the decline in thresholds at later time points may indicate a potential dose-dependent toxicity. Antiepileptic agents typically lower seizure threshold time during acute episodes by dampening neuronal hyperactivity [17].

Latency scores reveal that only the group pre-treated with low-dose DS exhibited a mild reduction in seizure duration compared to the positive control group (Table 3). This finding suggests an antiepileptic effect of DS, with the lower dose (60 mg/kg) being more effective than the higher dose (120 mg/kg). The observed reduction in seizure duration is consistent with the proposed mechanism of action for antiepileptic agents, which decrease neuronal hyper excitability and prolong seizure thresholds [18]. These potential

antiepileptic properties may be mediated by DS's bioactive compounds acting on inhibitory neurotransmitter pathways.

The elevated plus maze test revealed notable behavioural differences across treatment groups. The positive control group demonstrated anxiogenic traits, characterized by increased activity in the closed arms and reduced exploration of open arms. However, the group pre-treated with low-dose DS showed anxiolytic traits, spending significantly more time in the open arms. This supports earlier findings on the anxiolytic properties of DS rodent models [19]. Nonetheless, the group pre-treated with high-dose DS exhibited reduced activity in both open and closed arms, which may suggest toxicity at higher doses.

Short-term memory was evaluated using the Y-maze. Allowing the rat to explore all three arms of the maze allowed for the assessment of spontaneous alternation, a measure of spatial working memory that is fuelled by the animal's natural curiosity to explore previously unexplored locations. An animal with intact working memory and an intact neurological functioning, will recall the arms it has visited previously and will exhibit a propensity to enter an arm it hasn't visited as much. Spatial reference memory, which is supported by the hippocampus, can be assessed by training the test animals in the Y-maze with one arm closed. After an inter-trial interval of one hour, the animal should remember which arm it has not explored previously and visit it more often [20]. This study used the model mentioned above to assess the spatial reference memory of the animals because it has been reported that cognitive impairment is a common symptom of epilepsy. Some interrelated factors, such as the early onset of epilepsy, the frequency, severity, and length of seizures, as well as the use of AEDs, influence most cognitive issues [21]. The animals treated with DS extract, particularly the low-dose group, had significantly higher percentage alternation scores (Table 4) compared to the positive control group. The group treated with the carbamazepine also showed higher mean percentage alternation scores compared to the positive control group (Table 4). Success in the Y-maze test is indicated by a high rate of alternation, indicating that the animals can remember which arm was entered last [12]. The low-dose treatment group showed the highest cognitive function display, while the positive control group showed cognitive deficits. This result is in tandem with reports that epilepsy subjects also suffer cognitive decline [21].

Results of the TNF-α inflammatory test showed that the positive control group, which was given 300 mg/kg of isoniazid, only showed slightly elevated TNF scores than the treated groups (Table 5). The groups pre-treated with DS had lower mean TNF scores compared to the positive control group. This indicates that DS extract may possess an anti-inflammatory effect. Immune and inflammatory reactions have been documented to occur in various CNS diseases. Pro-inflammatory cytokine and associated molecule production are examples of inflammatory processes that have been observed in the brain following seizures in both clinical and experimental epilepsy cases. It has been suggested that some of the biochemical and structural alterations that take place during and after seizure activity may be mediated by activation of the innate immune system and related inflammatory processes in the brain, despite the fact that little is known about the function of inflammation in epilepsy [22].

Pre-treatment with DS extracts resulted in elevated SOD and catalase levels and reduced MDA levels compared to the positive control group (Table 5). Research reported that prolonged excitation of neurons caused by epileptic seizures leads to an increase in the concentration of reactive oxygen species, which may contribute to brain damage [23]. Hyperproduction of free oxygen radicals leads to disorders of intracellular calcium homeostasis, which modulates the excitability of neurons and synaptic transmission, making neurons more vulnerable and causing energy crashes and neuronal loss [24]. Another evidence of the effect of oxidative stress on the occurrence of epilepsy is the increased incidence of epileptic seizures in experimental animals [25]. The light microscopy study (figures 1-10) revealed that low-dose DS (figures 7 and 8) demonstrated neuroprotective effects against isoniazid-induced epilepsy, preserving hippocampal and amygdala cytoarchitecture. While the high-dose (figures 9 and 10) resulted in focal lesions, suggesting toxicity. Isoniazid alone (figures 3-4) caused severe neurodegeneration, characterized by cell loss and atrophy in the hippocampus and amygdala. Pre-treatment with low-dose DS (figures 7 and 8) effectively counteracted isoniazid-induced neurotoxicity, supporting its role as a potential neuroprotective agent.

Comparatively, carbamazepine (figures 5 and 6) offered partial protection with observable tissue abnormalities. This underscores the potentials of low-dose DS as an adjunctive anti-seizure therapy. Alkaloid extracts from the leaves modulated alterations of activities of critical enzymes of purinergic signalling (*in vitro* and *in vivo*), and the report suggested one of the mechanisms behind its neurological effects [26].

Research also reported that the phytoconstituents of DS improved motor coordination in haloperidol-induced cataleptic mice [27]. Also, magnesium and zinc oxide micronutrient components improved cognitive impairments and blood-brain barrier leakages [28]. The above reports correlate the neuroprotective abilities of plants from the family Solanaceae as observed in this study.

Antiepileptic, anxiolytic, and neuroprotective effects of DS observed in this study may be attributed to its phytochemical constituents, which include alkaloids such as scopolamine and atropine. These compounds are known to modulate cholinergic neurotransmission, which is critical in regulating neuronal excitability and anxiety-like behaviours. The potential mechanisms underlying the observed effects of DS may include modulation of GABAergic pathways [26] or interaction with ion channels involved in neuronal excitability [29]. Further studies involving detailed biochemical analyses are needed to elucidate the active compounds and pathways involved.

Despite the promising findings, this study has notable limitations. First, the lack of effect size reporting represents a key limitation that impacts the interpretive depth and translational potential of the study findings. Addressing this in subsequent research would strengthen the evidence base for the therapeutic use of DS. Second, the safety profile of DS is a source of concern. Its known toxicity, particularly at higher doses, raises questions about its translational potential as a therapeutic agent. Future studies should aim to isolate and purify the active components of DS to improve its safety profile, while also investigating its therapeutic window, long-term efficacy, and potential risks of adverse effects.

Conclusion

This study investigated the antiepileptic, anxiolytic, and neuroprotective effects of DS leaf extracts in isoniazid-induced epilepsy in Wistar rats. The low-dose extract demonstrated significant neuroprotection, reduced seizure duration, improved cognitive function, and mitigated oxidative stress and inflammation. However, the higher dose showed signs of toxicity, including focal lesions and reduced activity. These effects are attributed to phytochemicals like scopolamine and atropine, which may modulate GABAergic pathways and neuronal excitability. While the findings support the potential of DS as an adjunctive therapy for epilepsy, concerns about its toxicity and the lack of effect size reporting highlight the need for further research to isolate active components, refine dosing, and evaluate long-term safety and efficacy.

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