Neurprotective Role of Amaranthus Dubius on Penicillin Induced Experimental Epileptic Rat Model by Combating the Multineurotransmitter Deficiency

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ABSTRACT
Objective: The study aims to clarify the protective effects of Amaranthus dubius (AD) in penicillin (PCN)-induced experimental epilepsy models in rats. Method: In this study, twenty-four adult male Wistar albino rats, each weighing between 200-250 grams, were utilized. The rats were allocated into four groups: a control group, an Amaranthus dubius (AD) treated control group, a PCN-induced experimental epileptic rat model group, and an AD pretreated PCN-induced experimental epileptic rat model group. All groups were administered a daily oral dosage of 400 mg/kg body weight of AD aqueous leaf extract for fourteen days via an orogastric cannula. For the experimental epileptic model, PCN was injected into designated areas of the somatosensory cortex. On the concluding fifteenth day, subsequently, the rats were humanely euthanized. Tissue specimens from the cerebral cortex (CC), cerebellum (CB), caudate nucleus (CN), pons and medulla (PM), and midbrain (MB) were extracted, measured, and homogenized to facilitate subsequent biochemical analyses. Result: Pretreatment with AD has demonstrated significant changes in the levels of antioxidants and neurotransmitters in the brain. Conclusion: Amaranthus Dubius aids in combating multi-neurotransmitter deficiencies and enhances the body's antioxidant capabilities.

1. Introduction

Research on experimental models indicates a potential role for serotonergic transmission in epilepsy. The serotonergic system is intricate, encompassing at least 13 distinct G protein-coupled 5-HT receptors, plus one ligand-gated ion channel receptor (5-HT3), which are categorized into seven classes (5-HT1 to 5-HT7) (1).

Research has investigated the relationship between alterations in the brain's neurotransmitters, such as serotonin (5-HT), norepinephrine (NE), dopamine (DA), and endogenous activity levels, with conditions like depression and hyperactivity(2,3). Studies have shown that neurotransmitters can affect the immune system through molecular signaling(4). Furthermore, an overactive noradrenergic system, whether central or peripheral, has been associated with the development of anxiety and the presence of tremors (2,5).

Numerous knockout mouse models have established a connection between serotonin (5-HT), hippocampal malfunction, and the incidence of epilepsy. Mice deficient in the 5-HT1A receptor are more susceptible to seizures and exhibit a higher mortality rate following administration of kainic acid. These mice also show deficits in learning processes that depend on the hippocampus and display behaviors indicative of heightened anxiety—suggesting that the interplay between serotonergic systems and other neurotransmitters may be influential in shaping these behavioral outcomes(6). However, these observations are nuanced by the fact that 5-HT1A knockout mice also present with elevated dopamine levels and activity, as well as increased norepinephrine in the projection areas of the locus coeruleus, adding layers of complexity to the interpretation of these phenotypes(7).

Epidemiologists have observed a reciprocal relationship between the presumed dopaminergic overactivity in schizophrenia and the dopamine underactivity in epilepsy. This connection has led to the consideration of epilepsy as a condition of dopamine underactivity(8). Research also indicates that epileptic rats exhibit altered noradrenergic function. Experimental evidence supports the theory that the cortical noradrenergic system and related second messenger systems are compromised in epilepsy(9). Again, some researches have suggested an inverse correlation between noradrenergic activity in the brain and the susceptibility to seizures(10). However, Rutecki posits that norepinephrine may exhibit both proconvulsant and anticonvulsant properties(11).

Research indicates that oxidative stress, defined as the imbalance between free radical production and the body's antioxidant defense mechanisms, is significantly involved in the development of epilepsy (12,13). Enhanced

oxidative stress may result from excitatory neurotransmission, and is increasingly recognized as a potential factor in the causation of seizure-induced neuronal death(14). Additionally, epileptic seizures frequently occur in mitochondrial diseases caused by defects in oxidative phosphorylation(15).

Glutamate and related acidic amino acids are believed to be the primary excitatory neurotransmitters in the brain, potentially utilized by 40 percent of synapses. Therefore, two primary mechanisms—oxidative stress and the excessive activation of glutamate receptors—merge, representing sequential and interactive processes that lead to cellular vulnerability in the brain. The widespread presence of these processes that regulate oxidative stress and facilitate glutamatergic neurotransmission could explain their implication in a variety of disorders. However, the selective expression of these process components in specific neuronal systems might lead to targeted neurodegeneration in certain conditions (16). Topical administration of penicillins to animal brains has been shown to induce epileptiform convulsions, likely due to the antagonism of GABA-mediated inhibition(17).

Advancement in treating epilepsy is strongly connected to ongoing research into neuronal impulse transmission and the intricate aspects of neuron membrane structure and function(18). Billions of neurons in the brain communicate through chemical neurotransmitters like norepinephrine, serotonin, and dopamine, which are vital for signal transmission. Although the natural degeneration of brain cells is a part of aging, neurodegenerative diseases can hasten this decline(19). Epilepsy, a chronic and progressive neurological condition, is linked to persistent neuronal damage, particularly when not properly managed(20). Oxidative stress is believed to play a role in the onset and advancement of epilepsy, and therapies aimed at oxidative stress might lessen tissue damage and alter the course of the disease. Studies in vivo have demonstrated oxidative harm in animal epilepsy models and the success of antioxidant therapies in reducing such damage during epileptogenesis(2,3).

Plant-based foods are rich in vitamins and minerals that act as protective antioxidants. Beyond these, fruits, vegetables, nuts, seeds, and grains also contain thousands of other compounds that significantly contribute to our antioxidant intake. The antioxidant effect can be partially measured by 'ORAC,' which stands for Oxygen Radical Absorbance Capacity. ORAC scores are increasingly found on food and beverage packaging and in nutritional charts, aiding in the selection of antioxidant-rich foods for one's diet(21). While a diverse range of antioxidants in food plays a role in disease prevention, much of the research has concentrated on three particular antioxidants that are either essential nutrients or their precursors.

Amaranthus dubius (AD) is primarily recognized for its seeds and leaves, which are rich in proteins, amino acids, iron, dietary fibers, oils, and other micronutrients. Amaranths are utilized in treating various degenerative diseases due to their curative and protective attributes, which are linked to their remarkable antioxidant capacity. Numerous in vivo and in vitro studies support the high antioxidant potential of this versatile crop. Research into the availability of antioxidant-based phytonutrients is growing rapidly, emphasizing their beneficial chemical properties (22,23). AD leaves contain high levels of antioxidants (24). Aqueous extracts from red amaranth have shown significant anti-glycative effects, as well as anti-α-amylase, anti-α-glucosidase, anti-lipase, and anti-AchE activities (25). AD is also noted for modifying neurotransmitter levels in epileptic conditions due to its anti-inflammatory properties. Studies indicated that Amaranthus significantly enhanced learning and memory functions, reduced neurodegeneration, and lowered oxidative stress in the brain by boosting brain antioxidants (26).

So, our study aims to elucidate the protective role of AD on the PCN-induced experimental epileptic rat model.

2. Materials and methods

Animals used and their maintenance:

Forty-eight mature male Wistar albino rats of a pure strain, weighing 200–250 grams each, were used in the investigation. The rats were housed in a controlled laboratory setting prior to the experiments, following the rules set forth by the Institutional Ethical Committee. They were given free access to water and a standardized laboratory meal full of vital proteins, carbs, and minerals. Throughout the trial, the rats' body weight was frequently tracked and documented. Daily behavioral evaluations were conducted from 12:00 to 14:00.

AD Leaves water extract: Collection and preparation

Leaves of the AD plant, obtained from local markets, were authenticated, shade-dried, and finely pulverized. From this ground material, an extract was prepared, filtered, and then vacuum-dried at 40-50°C to obtain a dry powder. This powder was dissolved in double-distilled water to create the final formulation used in the

experimental study.

Treatment:

AD leaf extract was forcefully fed at a dose of 400 mg/kg body weight for fourteen consecutive days, between 10:00 and 11:00 hrs, using an orogastric cannula, following the lab's established standardization. Between 12:00 and 1:00 hrs., behavioral characteristics such seizure score, ictal phase, and interictal phase were recorded. The animals were put to death via cervical dislocation on the fifteenth day after therapy. The amounts of neurotransmitters and antioxidant enzymes were then assessed by harvesting brain regions such as the midbrain, medulla, pons, cerebellum, caudate nucleus, and cerebral cortex.

Grouping of Animal:

The research divided the rats into four separate groups, each consisting of 16 animals. The classifications were as follows: (1) the control group, (2) the cluster receiving only AD treatment, (3) the PCN-induced experimental epileptic rat model, and (4) the AD-pretreated PCN-induced experimental epileptic rat model.

Preparation of an experimental epileptic rat model using Penicillin::

Prior to starting the PCN-induced epileptogenesis treatment, participants fasted for a total of eighteen hours. In order to prepare for the surgical procedure, an intraperitoneal injection of sodium pentobarbital at a dosage of 40 mg/kg of body weight was used to administer anesthesia.

Topical application of penicillin (PCN):

The PCN solution was administered using a stereotaxic apparatus and a Hamilton syringe, ensuring a slow delivery of 0.05 ml (100 IU) over 5 minutes. The injection was aimed at precise locations within the somatosensory cortex—3.2 mm posterior to the bregma, 2.25 mm lateral to the midline, and 1 mm deep—perpendicular to the cortical surface. Following the injection, a three-day antibiotic prophylaxis regimen was implemented with intramuscular penicillin injections, each at a dosage of 10,000 IU (27). Control animals undergoing sham procedures received isotonic saline as a vehicle, in a volume equivalent to that used at the same site.

The progression of seizures in PCN-induced experimental epileptic animals was monitored at 15-minute intervals for 2 hours, following a modified protocol from Patel et al.(28) and Dybdal and Gale(29).

No response – ‘0’;

Gustatory movement / Fictive scratching – ‘1’;

Tremor – ‘2’;

Head bobbing – ‘3’;

Forelimb clonus – ‘4’

Rearing, falling, and clonus – ‘5’

Tissue preparation for the biochemical estimation:

On the fifteenth day, following the completion of the behavioral study, the animals were euthanized via cervical dislocation. The cerebral cortex (CC), cerebellum (CB), caudate nucleus (CN), as well as the pons and medulla (PM), and the midbrain (MB) were carefully excised.

Biochemical Estimation:

Estimation of antioxidant enzymes and lipid peroxidation:

(i) Tissue preparation:

The isolated brain tissue samples were precisely weighed, homogenized thoroughly in a chilled phosphate buffer, and systematically processed for subsequent biochemical analysis..

(ii) Measurement of Catalase (CAT):

Brain tissue samples were homogenized in phosphate buffer and then centrifuged at 3000 rpm for 10 minutes. The precipitate was agitated, and 9 ml of H_2O_2 was added. The rate of H_2O_2 decomposition was determined

spectrophotometrically by monitoring the absorbance change at 350 nm. Catalase activity was calculated using the percentage of inhibition units (30).

(iii) For Superoxide dismutase (SOD) measurement:

The brain tissue samples were homogenized in phosphate buffer and centrifuged at 3000 rpm for 10 minutes. Post-centrifugation, Trehalose-6,6-dibehenate (TDB) was added, followed by NADH to start the reaction. A solution of ethylenediaminetetraacetic acid and manganese chloride (EDTA-MnCl2) was then added. Spectrophotometric readings were taken at 340 nm, with a subsequent reading after adding Mercaptoethanol (31).

(iv) For Reduced glutathione (GSH) measurement:

An equal volume of homogenate was mixed with trichloroacetic acid (TCA) and centrifuged to separate proteins. The supernatant was treated with phosphate buffer, 5,5-dithiobis (2-nitrobenzoic acid), and distilled water. The mixture was vortexed, and the absorbance at 412 nm was measured within 15 minutes. The concentration of reduced glutathione was quantified and expressed as µg/g of tissue (32).

(v) Measurement of Lipid peroxidation (LPO):

Brain tissue samples were homogenized in a phosphate buffer and then centrifuged. The homogenate obtained was combined with TDB and incubated. Subsequently, trichloroacetic acid (TCA) was added, the mixture was vortexed, and the absorbance was measured at 350 nm. This was followed by a spectrophotometric reading and the addition of mercaptoethanol(27).

Estimation of brain neurotransmitters:

(i) Tissue Preparation:

Isolated brain tissues were washed with ice-cold saline and stored refrigerated for subsequent use. The tissues were homogenized in acidified butanol. Four milliliters of this homogenate were mixed with 10% heptane and 0.003N HCl, shaken for five minutes, and then centrifuged at 2000 rpm for ten minutes. Subsequently, 4.5ml of the acidic layer was extracted, combined with 200mg of alumina and 2M sodium acetate, shaken for five minutes, and centrifuged at 2000 rpm for another ten minutes. The supernatant obtained was used for serotonin (5-HT) estimation, while the precipitate was reserved for NE and DA estimation.

(ii) Serotonin (5-HT) Estimation:

To the supernatant, 10% isobutanal and boric acid buffer at pH 10 were added. Following shaking, 2 ml of 10% heptane was introduced. This was followed by the addition of 0.1N HCl, more shaking, and then 1ml of 0.3N HCl.

(iii) NE and DA Estimation:

The precipitate was mixed with cold distilled water and shaken thoroughly. It was then centrifuged at 2000 rpm for 30 seconds. 0.33N acetic acid was added and the mixture was centrifuged again at 2000 rpm for 3 minutes. The supernatant was transferred to a glass-stoppered centrifuge tube. The acid elution process was repeated. A freshly prepared mixture of ethylene diamine and ethylene diammonium dihydrochloride (7:5) was added and incubated at 50°C for 40 minutes. After cooling to room temperature and saturating with sodium chloride, isobutanol was added. The mixture was centrifuged at 2000 rpm for 30 minutes. The supernatant was collected for DA estimation, and the precipitate was processed with cold distilled water for NE estimation. A Perkin-Elmer Spectrofluorometer was utilized to measure the fluorescence of all samples. The excitation and emission wavelengths for 5-HT were 295 and 550nm, for NE 385nm, and for DA 320 and 370nm, respectively (33).

Statistical analysis:

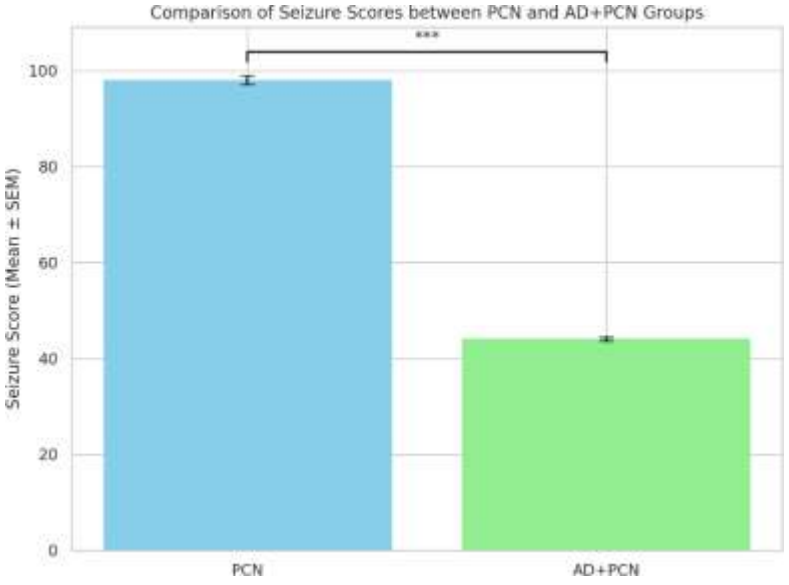
The data were presented as the mean ± SEM and statistically analyzed (one-way ANOVA, F – statistic, P- value) along with the Coefficient of Variation (CV). Furthermore, the data scaling was conducted to enhance visualization and comparison, involving the transformation of data to fit within a specific scale, typically between 0 and 1. This method is beneficial for comparing various groups or measurements on a uniform scale, particularly when the original values have a wide range.

3. RESULTS

Fourteen days after administering PCN, the levels of brain antioxidants, and neurotransmitters were assessed. Pretreatment with AD leaf extract markedly changed the levels of these endogenous antioxidants and neurotransmitter levels.

The study's findings indicate that the treatment resulted in a substantial decrease in seizure scores by 54.0 units, offering a considerable degree of seizure protection. This is evidenced by the 52% protection rate observed in the AD+PCN group when contrasted with the PCN group alone.

Fig. 1: Seizure scores between the PCN group and AD pretreated group.



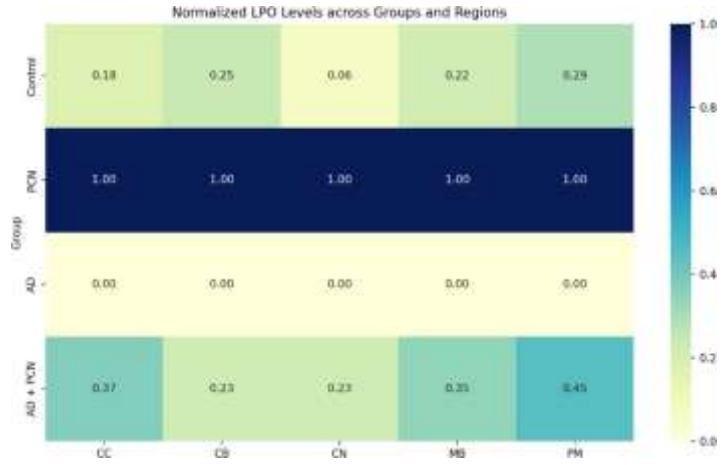
The post-hoc t-test comparing the PCN group and the AD+PCN group yielded a T-statistic of approximately 53.61 and a p-value of \(1.23 \times 10^{-13}\), signifying a highly significant difference. These results demonstrate a substantial and significant difference in seizure scores between the two groups, implying that the AD+PCN treatment may have a considerable effect compared to the PCN-induced group.

Measurement of brain antioxidant levels:

LPO level:

The scaling clarifies the relative differences between measurements: The PCN group exhibits the highest values (1.0) in all regions, the AD group presents the lowest values (0.0) in all regions, and the Control and AD + PCN groups display intermediate values. Consequently, the relative patterns across various regions are discernible.

Fig 2: LPO levels

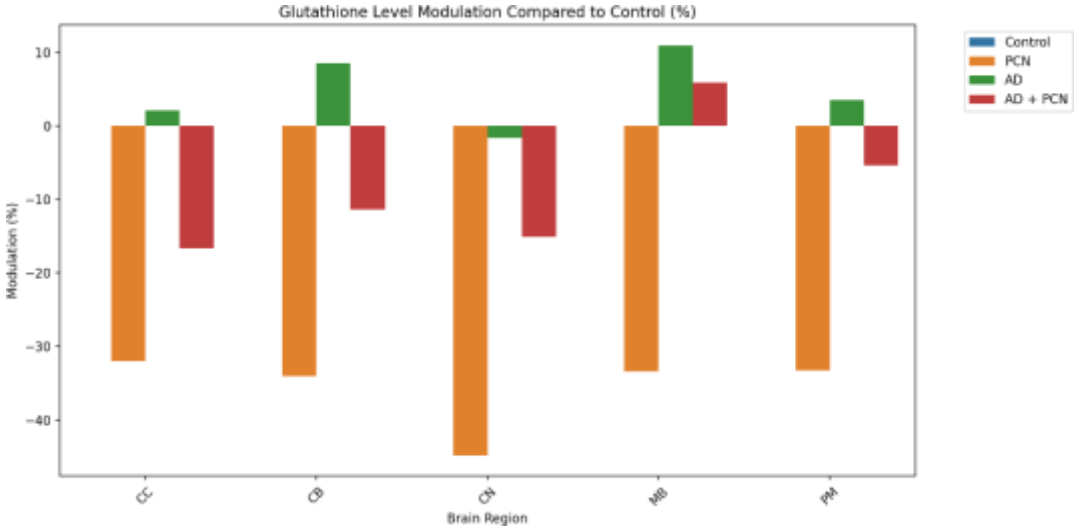


This scaling reveals the relative differences between measurements more clearly:

The PCN group exhibits the highest values, scoring 1.0 across all regions. Conversely, the AD-pretreated group registers the lowest values at 0.0 in every region. Meanwhile, the Control and AD + PCN groups display intermediate values.

GSH level:

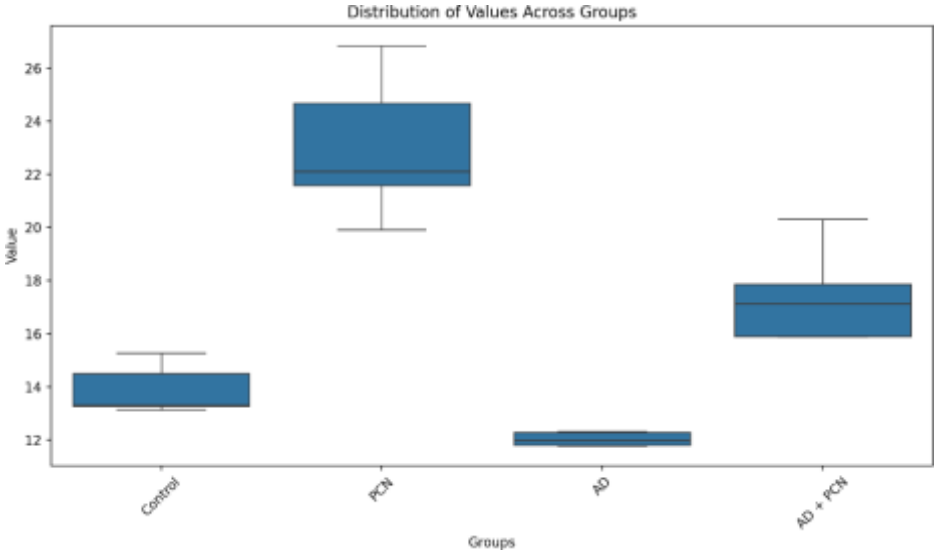
Fig 3: GSH levels



An F-statistic of 47.3352 indicates a significant difference, while a p-value of 0.0000 (commonly reported as < 0.0001) reinforces this finding. These statistics imply that the reduced glutathione levels significantly vary among the different groups in the study.

SOD:

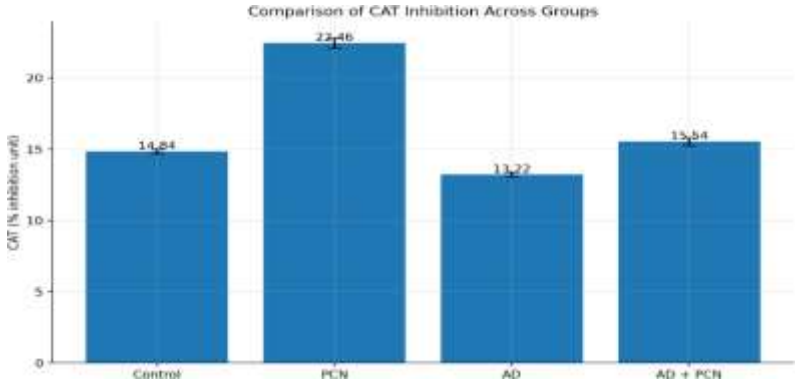
Fig 4: SOD levels



An F-statistic of 39.6636 and a p-value of 1.2299e-07 suggest significant differences.

CAT:

Fig 5: CAT levels



F-statistic: 141.71321602396165 (An F-statistic of approximately 141.71 indicates significant differences.)

p-value: 9.835168471784194e-12 (A very small p-value, roughly 9.84e-12 or \(9.84 \times 10^{-12}\), provides strong evidence against the null hypothesis. A p-value below 0.05 is generally regarded as statistically significant, and in this case, the p-value is well beneath that threshold).

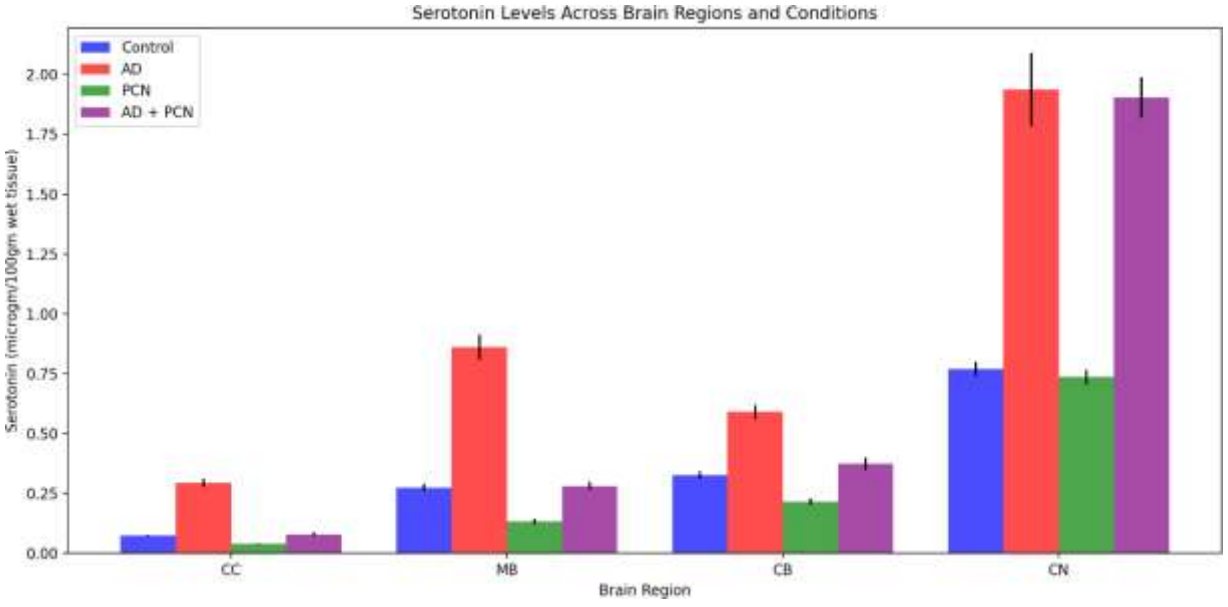
These results suggest that there are significant differences in the mean values of the groups being compared (Control, PCN, AD, AD + PCN).

Measurement of brain monoamines

Changes in the serotonin (5-HT), dopamine (DA) and Norepinephrine (NE) levels

In animals with epilepsy treated with AD, there was a significant increase in serotonin (5-HT) levels in the cerebral cortex, cerebellum, caudate nucleus, and midbrain. Conversely, dopamine (DA) levels decreased across all cortical areas, with the exception of the CN, when compared to epileptic control groups. Additionally, AD treatment markedly raised norepinephrine (NE) levels in the CC, MB, and CN, while a decrease was observed in the CB (Fig6,7 and 8).

Fig 6: Serotonin levels



The ANOVA tests were successfully conducted for each region and overall, with significant F-statistics and p-values indicating differences among the groups. The previous errors were resolved by correctly handling the

data and ensuring proper input for the ANOVA function. Here are the results:

One-way ANOVA Results for Each Region:

Region: CC

F-statistic: 1359.4163; p-value: 2.8286e-23

Region: MB

F-statistic: 655.4423; p-value: 3.9447e-20

Region: CB

F-statistic: 216.0215; p-value: 2.1103e-15

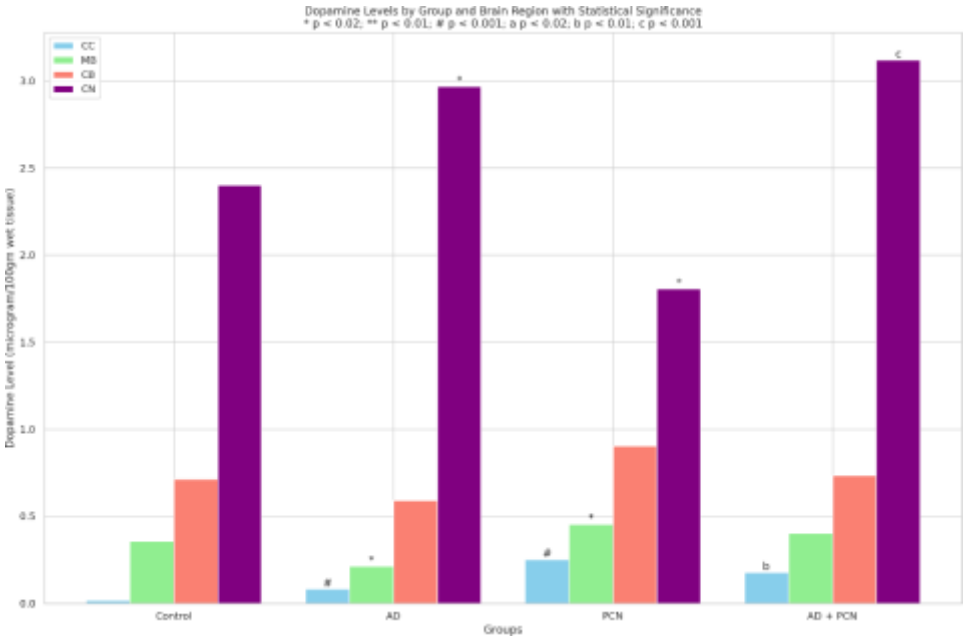
Region: CN

F-statistic: 388.6106; p-value: 6.8380e-18

Overall ANOVA Results:

An F-statistic of 979.7320 suggests a significant difference between the group means under comparison, which is statistically significant. With a p-value of 5.2250e-84, well below the standard significance thresholds like 0.05 or 0.01, the results are confirmed to be statistically significant.

Fig 7: Dopamine levels



Based on the one-way ANOVA analysis for each brain region:

Cerebral Cortex (CC):

F-statistic: 613.74; P-value: 7.56e-20 (extremely significant)

Midbrain (MB):

F-statistic: 822.86; P-value: 4.14e-21 (extremely significant)

Cerebellum (CB):

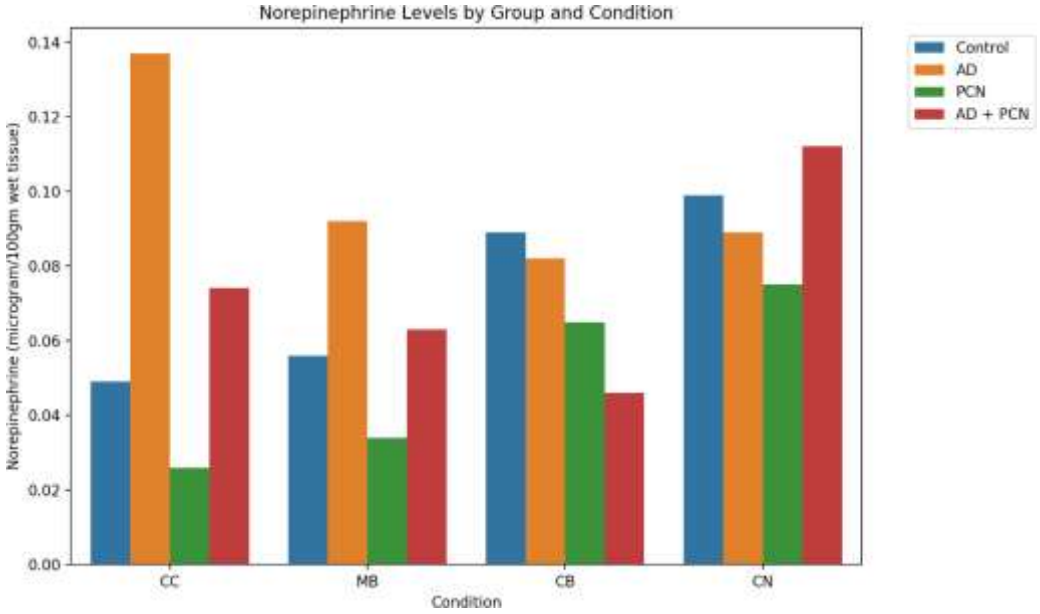
F-statistic: 1084.34; P-value: 2.68e-22 (extremely significant)

Caudate Nucleus (CN):

F-statistic: 23900.28;P-value: 1.05e-35 (extremely significant)

Statistical analysis reveals significant differences in all brain regions between groups ($p < 0.001$), demonstrating that the treatments (AD, PCN, and AD+PCN) significantly impact dopamine levels when compared to the control group. The notably high F-statistics, especially in the CN region, indicate exceptionally strong effects of the treatments in comparison to the variance within the groups.

Fig 8: Norepinephrine levels



The mean and SEM for each group and condition have yielded significant F-statistics and p-values for each condition, indicating notable differences between groups. Subsequent post-hoc t-tests have pinpointed the specific differences among groups. Below are the results:

CC:

F-statistic: 143.4030; p-value: 0.0000

Post-hoc t-tests:

Control vs AD: p = 0.0000; Control vs PCN: p = 0.0000; Control vs AD + PCN: p = 0.0001;

AD vs PCN: p = 0.0000; AD vs AD + PCN: p = 0.0000; PCN vs AD + PCN: p = 0.0000

MB:

F-statistic: 116.2658; p-value: 0.0000

Post-hoc t-tests:

Control vs AD: p = 0.0000; Control vs PCN: p = 0.0000; AD vs PCN: p = 0.0000;

AD vs AD + PCN: p = 0.0000; PCN vs AD + PCN: p = 0.0000

CB:

F-statistic: 35.7748; p-value: 0.0000

Post-hoc t-tests:

Control vs AD: p = 0.0307; Control vs PCN: p = 0.0011; Control vs AD + PCN: p = 0.0000;

AD vs PCN: p = 0.0234; AD vs AD + PCN: p = 0.0000; PCN vs AD + PCN: p = 0.0046

CN:

F-statistic: 22.0559; p-value: 0.0000

Post-hoc t-tests:

Control vs AD: p = 0.0037; Control vs PCN: p = 0.0001, AD vs PCN: p = 0.0364;

AD vs AD + PCN: p = 0.0011; PCN vs AD + PCN: p = 0.0000

The findings indicate substantial variations in norepinephrine levels among various conditions and groups. The data imply that there are significant differences in norepinephrine levels between different treatment groups and conditions.

4. Discussion

In the PCN-induced group, it was observed that serotonin (5-HT) levels in various brain regions, namely the cerebral cortex (CC), cerebellum (CB), caudate nucleus (CN), midbrain (MB), and pons and medulla (PM), significantly increased in the AD-pretreated group (fig.6). Conversely, dopamine (DA) and norepinephrine (NE) levels significantly decreased in the CC, CB, CN, MB, and PM (fig.7) compared to the control group. However, in PCN-induced epileptic animals, the 5-HT levels in the same brain regions significantly decreased compared to the AD-pretreated group, while DA and NE levels significantly increased in the CC, CB, CN, MB, and PM. AD-pretreated, PCN-induced experimental animals exhibited a significant increase in 5-HT levels across these brain regions (Fig.8) and a significant decrease in DA and NE levels in the CC, CB, CN, MB, and PM when compared to the PCN-induced experimental model.

In epileptic animals, the likely cause is the activation of 5-HT1A autoreceptors, which inhibit serotonergic neurons, resulting in reduced 5-HT release and neurotransmission. Central 5-HT1A receptors serve as somatodendritic presynaptic autoreceptors in the raphe nuclei and as postsynaptic receptors in areas like the hippocampus, where they may exhibit varying functional and regulatory characteristics depending on the structure they innervate (34,35).

Activation of 5-HT1A receptors leads to a hyperpolarizing response in the membrane due to an increase in potassium conductance(36,37), showing anticonvulsant effects in various experimental seizure models, both in vivo and in vitro. These models include hippocampal kindled seizures in cats, seizures in rats induced by intrahippocampal kainic acid, and seizures triggered by picrotoxin, bicuculline, and kainic acid in rat hippocampal slice preparations(38). The anticonvulsant effects of activating 5-HT1A receptors differ across various regions and models(39).

Alterations in the brain's neurotransmitters, such as serotonin (5-HT), norepinephrine (NE), and dopamine (DA), correlate with endogenous activity levels, leading to conditions like depression or hyperactivity (40, 33). Noradrenergic hyperactivity, whether central or peripheral, is implicated in the development of anxiety and tremors (40, 41). Epidemiologists have observed a reciprocal relationship between the presumed dopaminergic hyperactivity in schizophrenia and epilepsy, which is now considered a condition of dopamine hypoactivity. The identification of various dopamine receptor families (D1 and D2), which exert opposite effects on neuronal excitability, has marked the advent of a new phase in dopamine-epilepsy research (42).

This study showed that epileptic rats have reduced noradrenergic function. Pretreatment with AD leaf extract showed significant improvements in the PCN-induced epileptic animals. Thus, the antioxidants and the flavonoids of the AD leaf extract helped to improve the epileptic condition by altereing the brain monoamines. Hence, from the present observation, it can be concluded that Amaranthus Dubius has a neuroprotective role against Penicillin induced Epilepsy.

Experimental evidence indicates that an increase in norepinephrine (NE) leads to immunosuppression, as studies have shown NE suppresses lymphocyte function (43). Additionally, dopamine (DA), a monoamine catecholamine neurotransmitter with central nervous system functions, has been identified as a critical regulator of various physiological functions in the periphery, including a regulatory role on the immune system. This interaction between the nervous and immune systems is crucial in determining the immune response. DA can directly regulate immune effector cells and, when necessary, can down-regulate the inhibition of effector T lymphocyte proliferation without affecting TNF-α or IFN-γ production (44). Similarly, serotonin (5-HT) plays a pivotal role in modulating immune cells and both innate and acquired immunity (45). Our results suggest that alterations in the levels of 5-HT, DA, and NE lead to changes in brain antioxidant levels during epilepsy in experimental epileptic animals (PCN), but pretreatment with AD reverses the changes in neurotransmitter levels boosting the brain antioxidant levels.

5. Conclusion

This study indicates that AD plays a crucial role in combatting the multi neurotransmitter deficiency. The findings suggest that AD alters neurotransmitter levels, enhances the immune system, and boosts the body's antioxidant capacity, offering a potential herbal remedy for managing epilepsy.

Future Scope of the Study

Identifying the active constituent of AD is crucial, and it is key to carry out further research to assess its therapeutic potential. Additionally, human clinical trials are necessary to evaluate the effectiveness of AD.

Conflict of Interests

There are no conflicts of interest.

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