EFFICACY OF ASCORBIC ACID ON FRONTAL CORTEX DAMAGE INDUCED BY ALCHOLIC EXRACT OF *DATURA STRAMONIUM* LEAF IN ADULT MALE WISTAR RATS.

ABSTRACT

Context

Datura stramonium (DS) is a medicinal plant widely distributed across the globe, including in Nigeria and West Africa. Pharmacological, physiological, and histological studies have demonstrated the neurotoxicity of the plant in animals and humans. Ascorbic acid is *a potent reducing agent and scavenger of free radicals in biological systems.*

Aim

This study was undertaken to advance our knowledge on Datura stramonium leaf toxicity and investigate ascorbic acid efficacy on the frontal cortex damage induced by the alcoholic extract of Datura stramonium leaf in adult male Wistar rats.

Settings and Design

Thirty (30) adults male Wistar rats weighing about 120±20g were divided into six groups (A-F) of five animals each for oral administration over 14 days.

Materials and Methods

- ***Group A (Control)**: Received only rat feeds and water.*
- ***Group B**: Received 200 mg/kg alcoholic extract of Datura stramonium.*
- ***Group C**: Received 400 mg/kg alcoholic extract of Datura stramonium.*
- ***Group D**: Received 200 mg/kg body weight alcoholic extract of Datura stramonium and 200 mg/kg body weight of Vitamin C.*
- ***Group E**: Received 400 mg/kg body weight alcoholic extract of Datura stramonium and 200 mg/kg body weight of Vitamin C.*
- ***Group F**: Received 200 mg/kg body weight of Vitamin C. The experimental animals were euthanized, and sections of the frontal cortex of the brain were harvested for histological procedures, organ weight (brain) and body weight of experimental animals were obtained.*

Statistical Analysis

The data was subjected to a one-way analysis of variance (ANOVA).

Results

Histological observations indicated that the administration of the alcoholic extract of DS leaf in Group C showed degeneration of neurons in the frontal cortex. Groups that received ascorbic acid along with

DS leaf in smaller doses showed no significant changes and had normal neuronal cells and stroma. Changes observed in body weight were not statistically significant at p-value <0.05.

Conclusion

This study suggests that ascorbic acid effectively reduces the neurotoxicity potential of Datura stramonium on the frontal cortex. The DS leaf extract may have neurodegenerative effects at high doses, and precautions should be taken when consuming DS, as it may adversely affect and damage neurons in the frontal cortex.

Keywords*: Datura stramonium, Vitamin C, Neurodegeneration, Frontal cortex neuron.*

1. INTRODUCTION

Medicinal plants are an important source of therapeutic compounds used in treatment of many diseases since ancient times (Singh *et al*., 2022). Medicinal plants are often used as raw materials for extraction of active ingredients which are used in the synthesis of different drugs and they have demonstrated several pharmacological activities such as anti-inflammation, anti-cancer, anti-allergic, and antimicrobial infection (Sharma et al., 2021; Shakya, 2016).

Various parts of medicinal plants, such as leaves, roots, bark, fruit, seeds, and flowers, contain phytochemicals responsible for their biological activities and can be processed into new drugs (Ugboko et al., 2020).

Numerous scientific reports suggest that medicinal plants could serve as a promising alternative to ineffective antibiotics in combating infectious diseases (Abdallah et al., 2023). Among them, Datura stramonium is a highly important plant due to its high content of tropane alkaloids and its traditional medicinal use throughout the world (Batool et al., 2020).

Datura stramonium is a shrub, a wild-growing plant of the Solanaceae family, distributed all over the world including Nigeria in West Africa (Mohanlall, 2020). It is commonly known as Devils Snare, belongs to the Solanaceae (night shade) family. It is also known by the common name Jimson weed. Other common names for *D. stramonium* include thorn apple and moon-flower, it is popularly called GEGEMU OR EWE KAN by the Yoruba tribe of Nigeria. It is believed the plant has been an exemplary source of folklore medicinal herb known for its mental stimulation and curative properties (Choudhary et al., 2021). Recently, it has been used as narcotic and local anesthetic drug in many societies and in some nations young people use the leaves by smoking for hallucination purpose. Addiction by the youths who are more prone to dangers of smoking and drug abuse use

this plant. Datura Stramonium plant should only be used therapeutically under the care of knowledgeable health care professionals. The adverse effects can be extremely severe and detrimental. Therefore, even in light of its many beneficial effects, the risk-benefit ratio should always be taken into consideration before the usage (Firdaus et al., 2020).

Datura contains different types of phytochemical including saponins, tannins, steroids, flavonoids, phenols and glycoside. It contains a variety of tropane alkaloids such as atropine, hyoscyamine, and scopolamine, which have been reported to induce oxidative stress in different systems and tissues.

Fig 1 *Datura stramonium* Source; Monaco nature encylopedia

1.2 Phytochemical Constituent of D*atura Stramonium*

Datura stramonium contains sixty-four different types of tropane alkaloid. The major tropane alkaloids: hyoscyamine and scopolamine and several minor tropane have been identified in *Datura* species. The primary biologically active substances in *D. stramonium* are the alkaloids atropine and scopolamine. The aqueous and the ethanolic extract of the stem bark of Datura stramonium contain

many phytoconstituents such as alkaloids, saponins, tannin, steroids, flavonoids, phenols, steroidal glycosides, amino acids, carbohydrates and terpenoids (Srivastava and Srivastava 2020; Batool et al., 2020).

1.3 Pharmacological Actions

Datura is known to exhibit analgesic, antioxidant, anticancer, and antimicrobial properties.

- **Anti-inflammatory and Analgesic Activities**

The phytochemicals present in Datura species are well-known for their anti-inflammatory and analgesic properties due to their ability to suppress the production of chemical mediators responsible for the stimulation of nociceptors and induction of pain or inflammation (Sharma *et al.*, 2021).

- **Antioxidant Activities**

The antioxidant activity of Datura extracts can also be attributed to the presence of phytochemical compounds, which acts as potent free radical scavengers and help prevent cellular damage. Analysis of *Datura* plant extracts for antioxidant characteristics revealed its ability to cure various health disorders, including cancers, cell damage, aging and several other disease (Sharma *et al.,* 2021).

- **Antimicrobial Activities**

The antimicrobial activity against pathogenic microbes was evaluated using aqueous and ethanolic extracts of different parts of *D. stramonium* and the results revealed that the ethanolic extracts showed better antimicrobial activity than the aqueous extracts. Moreover, the leaf extract were found to be more effective than stem and root.

- **Anti-Asthmatic Activities**

D. stramonium contains a variety of alkaloids including atropine and scopolamine, having an anticholinergic and broncho dilating activity. Atropine and scopolamine act on the muscarinic receptors by blocking them (particularly the M2 receptors) on airway smooth muscle and submucosal gland cells, which dilate bronchial smooth muscle and ease asthmatic attacks (Srivastava and Srivastava 2020).

- **Anticancer Activities**

D. stramonium was reported to have anticancer effect against human epidermal carcinoma of the nasopharynx at a therapeutic dose of 0.05 to 0.1g. However, precaution should be taken while using *Datura* as an anticancer agent since adverse anticholinergic effects may occur (Srivastava and Srivastava 2020).

1.4 Toxicity of Datura Stramonium

All parts of Datura plants contain dangerous levels of tropane alkaloids, which are classified as deliriants or anticholinergics. Tropane alkaloids are neurotoxic, and their frequent recreational abuse may lead to delirium and death (Choudhary et al., 2021). Symptoms of toxicity include fever, dry skin, dry mouth, headache, hallucination, convulsions, rapid and weak pulse, acute confusion, tachycardia, coma, and death. Intoxication with Datura extract can adversely impact the central nervous system, leading to disorientation, memory loss, impaired vision, hyperpyrexia, and respiratory and cardiovascular issues (Sharma et al., 2021; Mukhtar et al., 2019).

1.5 Uses of Datura Stramonium

- **Traditional Use**

In Ayurvedic medicine, *Datura stramonium* is described as a useful remedy for various human ailments including ulcers, wounds, inflammation, rheumatism and gout, sciatica, bruises, and swellings, fever, asthma, bronchitis and toothache (Mohanlall and Ally 2020 ; Sharma *et al.,* 2021).

Holzman (2021) used extracts from Datura and morphine to induce twilight sleep treatment for women experiencing difficult childbirth.

- **Medicinal Use**

Datura has been used since ancient times for asthma symptoms. Its leaves are used for their antispasmodic, hypnotic, and narcotic properties (Shekhar et al., 2017). Hindu physicians describe its usefulness in treating fever, skin diseases, boils, itch, worms, and insanity. Findings show that Datura stramonium oil can be an immunotherapeutic agent for colon cancer treatment (Akbar & Akbar, 2020; Chandan et al., 2020). Atropine and scopolamine in Datura have Parkinson's disease-related medicinal potency, including the treatment of motor sickness and bradycardia (Lawal et al., 2023).

1.6 Aim of Study

The aim of the study is to evaluate the effects of ascorbic acid on frontal cortex damage induced by alcoholic extract of *Datura stramonium* leaf in adult male wistar rat.

1.7 Significance of the Study

This study was undertaken to advance our knowledge of the toxicology of Datura stramonium leaf in adult male Wistar rats, the histo-morphological effect of Datura stramonium leaf on the frontal cortices in adult male Wistar rats, and the effect of ascorbic acid on the frontal cortex in adult male Wistar rats.

2.0 VITAMIN C (ASCORBIC ACID)

Vitamin C is a naturally occurring organic compound with antioxidant properties, found in both plants and animals. Vitamin C is one of the potent reducing agents and scavengers of free radicals in biological systems and harmful oxygen-derived species such as hydroxyl radical, hydrogen peroxide and singlet oxygen and its implicated in many diseases of free radical pathology, including biomolecular-, cellular- and tissue damage-related diseases, as well as cancer and ageing. It is a cofactor for enzymes involved in regulating photosynthesis, hormone biosynthesis, immune stimulation, neurotransmitters, synthesis of collagen and regenerating other antioxidants which also regulates cell division and growth, and also has roles in detoxifying the body of heavy metals. Ascorbate forms a complex with Fe3+ followed by reduction to Fe2+, which may potentiate free radical production (Kontoghiorghes, 2020).

Vitamin C also plays an important role in abiotic stress tolerance, and considerable interest as been on it due to its ability to induce a protective effect on plants under stress. It has been supported that vitamin C induced increase in the resistance of plants on heavy metal stress (Pehlivan, 2014).

2.1 Redox Metabolism and Antioxidant Properties of Vitamin C

Oxidative stress occurs due to the imbalance between the availability of antioxidants and the production of reactive oxygen species (ROS) in favor of the latter. This imbalance leads to the disruption of mitochondrial function. It contributes to neuronal degeneration, characterized by the

progressive loss of neuron cells, compromised motor or cognitive functions, and the accumulation of abnormally aggregated proteins, which can lead to neurodegenerative diseases (Kowalczyk, 2021; Cenini et al., 2019). Excess ROS can either oxidize biomolecules or structurally modify proteins and genes, triggering signaling cascades that can lead to the onset and progression of inflammatory diseases. Reflexively, enhanced ROS generation by immune cells at the site of inflammation causes oxidative stress and tissue injury (Chatterjee, 2016).

The oxidation process is a chemical reaction that produces free radicals, leading to chain reactions that damage cells (Pehlivan, 2017). Vitamin C is a powerful antioxidant that can donate a hydrogen atom and form a relatively stable ascorbyl-free radical. It is suggested to decrease oxidative damage and lower the risk of certain chronic diseases. Vitamin C (L-ascorbic acid or ascorbate) is a biomolecule in many biochemical processes. It has various bodily functions, making it an essential antioxidant by nature and a pro-oxidant (Akbari, 2016).

Antioxidant compounds, at low concentrations, delay or prevent the oxidation of a substance and act through several chemical mechanisms: hydrogen atom transfer (HAT), single electron transfer (SET), and the ability to chelate transition metals (Santos-Sánchez, 2019).

2.2 Role of Vitamin C (ascorbic acid) in Lipid Peroxidation

The chemical and biological properties of L-ascorbic acid suggest that it can act as an antioxidant in vivo. Vitamin C is a primary antioxidant in that it directly neutralizes radical species. It is not very active with prevalent cellular oxidants such as hydrogen peroxide and probably reacts with hydrogen peroxide breakdown products. Vitamin C has the ability to protect against lipid peroxidation by acting as a scavenger of ROS and by one-electron reduction of lipid hydroperoxyl radicals via the Vitamin E redox cycle (Pehlivan, 2017).

2.3 Ascorbic Acid in Human Disease

Vitamin C could be involved in several disease processes as an electron donor. It is present in almost all foods of plant origin. The mineral vitamin C requirement for humans is 40-60 mg/day to combat dietary deficiency. However, Vitamin C status decreases with both age and smoking and is associated with chronic diseases such as rheumatoid arthritis and cancer. Vitamin C consumption prevents free radical-induced damage to DNA, which is thought to be an initiating step in cancer formation.

The brain performs various functions, including receiving information from and controlling the activities of the trunk and limbs, mainly through its connections with the spinal cord. It also receives information and controls the activities of the head and neck structures through cranial nerves. Additionally, it is responsible for personality, thoughts, and aspirations (Singh, 2020).The brain is metabolically one of the most active organs in the body and is highly susceptible to free radical attack and oxidative stress. Datura stramonium exerts neurotoxic effects by increasing oxidative stress (Igben et al., 2023), while ascorbic acid delays or prevents oxidative stress.

In recent years, research has been conducted to investigate the effects of Datura stramonium on the frontal cortex. Intake of large doses of Datura stramonium has effects on the central nervous system and produces symptoms like confusion, hallucinations, and amnesia.

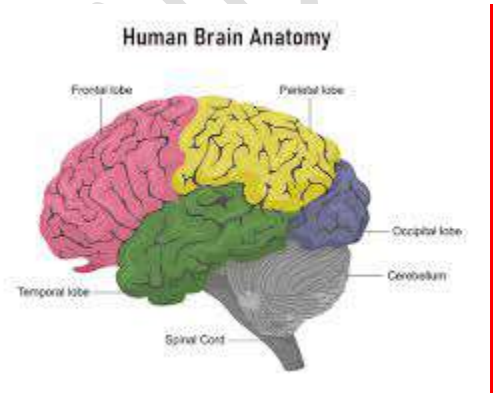


Figure 2: Structure of the brain. Source: John Hopkins Medicine

3.0 MATERIALS AND METHODOLOGY

3.1: Materials

The following materials were utilized in the experiment:

- Datura stramonium
- Vitamin C
- 30 Wistar rats
- Widen cages
- Water plates
- Feeding plates
- Standard rat feed and waterS
- Weighing scale
- Oral cannula
- Syringes and needles
- Dissecting gloves
- Dissecting sets and slab
- Measuring cylinder
- Fixatives
- Hematoxylin and eosin
- Xylene and paraffin wax
- Glass slides and cover slips
- Graded alcohol
- DPX mountant
- Cotton wool
- Staining jars
- Tissue cassettes and molds
- Heater
- Microscope

3.1.2Site of Study

The study was conducted in the animal house of the Anatomy Department at the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State.

3.2 Methodology

3.2.1 Preparation of Ethanolic Extract of *Datura stramonium* Leaf

Fresh Datura stramonium leaves were collected from Pectorial Guest House, Oke Anu, Ogbomoso, Oyo State, Nigeria. The leaves were identified at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, with the voucher numbers LH0731 and LH0588. The leaves were air-dried for two weeks, then pounded into powder form using a mortar and pestle. The powder was measured with a metal-sensitive weighing balance and soaked in distilled water for three days. The mixture was sieved using a muslin cloth, and the filtrate was collected and processed at the Department of Food Science and Technology, LAUTECH, where the dried sample was obtained (Gad-Elkareem et al., 2019).

3.2.2 Acclimatization of the Experimental Animals

Thirty male wistar rats, with an average body weight of 120 grams, were obtained from the Department of Anatomy animal house at LAUTECH. The rats were acclimatized for two weeks and weighed weekly during this period and throughout the three-week experimental period. The animals were well-handled and maintained at a constant room temperature of 25°C. They were provided with standard rat feed and water *ad libitum.*

3.2.3 Animal Sacrifice and Collection of Organs

The experimental animals were sacrificed via cervical dislocation. The skulls were thoroughly dissected, and the brains were identified and harvested. Blood samples were also collected for further studies. The brains were then processed for tissue analysis.

3.3 EXPERIMENTAL DESIGN

The rats were randomly divided into six groups: A, B, C, D, E, and F (n=5 rats each).

- **Group A**: The control group was administered standard rat feed ad libitum.
- **Group B**: Administered 200 mg/kg body weight of *Datura stramonium* extract orally every day for 14 days.
- **Group C**: Administered 400 mg/kg body weight of *Datura stramonium* extract orally every day for 14 days.
- **Group D**: Received 200 mg/kg of Datura stramonium extract and 200 mg/kg body weight of vitamin C orally daily for 14 days.
- **Group E**: Received 400 mg/kg of Datura stramonium extract and 200 mg/kg body weight of vitamin C orally daily for 14 days.
- **Group F**: Received 200 mg/kg body weight of vitamin C orally daily for 14 days.

At the end of the second week, the animals were sacrificed by cervical dislocation, and their brains were immediately removed.

The LD50 of ethanolic extract of *Datura stramonium* leaf was reported to be 3185.25 mg/kg in rats (Al-snafi, 2017).

The LD50 of Vitamin C was reported to be 11900 mg/kg in rats (Ghaleb et al., 2019; Animoku et al., 2019).

3.3.1 Histological Procedures

3.3.2 Tissue Processing

Thin slices were taken from each brain and placed in a tissue cassette, which was then placed in tissue baskets. An automated tissue processor was used to carry out the stages involved in tissue processing, including fixation, dehydration, clearing, infiltration, embedding, sectioning, floating, drying, staining, and mounting (Nayak, 2017).

3.3.3 Fixation

The brain was fixed in formal calcium, which is obtained by 40% formaldehyde (100 ml), calcium chloride (10g), and Distilled water (900 ml) (Bhat & Hussein, 2021). This was used to preserve the natural tissue structure and maintain the cell structure from degradation.

3.3.4 Dehydration

After fixation, the tissues were removed and rinsed in running water before being subjected to dehydration. This involves removing water from the fixed tissue, which is removed from the tissues through the dehydration method through ethanol(Gartner, 2020).

3.3.5 Clearing

The tissue is now water-free, but wax infiltration cannot occur because wax and ethanol are immiscible. Therefore, an intermediate solvent fully miscible with ethanol and paraffin wax is used. The solvent used for these intermediate stages is usually xylene(O'Dowd et al., 2023). The tissue was placed in two changes of xylene for 1 hour each (MD, 2019); the ethanol was gradually replaced with xylene, and when the tissue was embedded, the xylene was replaced by the molten paraffin wax (Gunasegaran, 2016).

3.3.6 Infiltration

This involves transferring tissues into a bath of molten paraffin in one of the chambers of the automatic embedding machine twice for 1 hour each (MD, 2019), where they solidify to a constituent that allows sections to be consistently cut. Tissue is used for this process.

3.3.7 Embedding

In staining, the process of embedding is done using paraffin wax to enhance easier extraction of cellular structures. In complex cellular tissues, plastic resin wax or a combination of fixatives produces good morphology (Alturkistani et al., 2016). The specimen is thoroughly infiltrated with wax and formed into a block, which can be clamped into a microtome for section cutting. This step uses an "embedding center," where a mold is fixed with molten wax, and the specimen is placed.

The specimen is very carefully oriented in the mood because its placement will determine "the planes of the section" (Dey,2018).

3.3.8 Sectioning and Floating

Sectioning refers to the preparation of "ribbon" like microtomes of tissue to mount on a microscope slide for examination (Knoblaugh et al., 2021). Tissue is embedded with optimal cutting temperature (OCT) or paraffin before being sectioned. This is achieved by using a machine called a microtome to cut the tissue into the desired planes (longitudinal, transverse, sagittal) and desired thickness (Winsor & Sluys, 2018). When the section has fully expanded and flattened on the surface of the warm water, it is picked up with a well-labeled slide and transferred into an incubator (45-50) for at least 1 hour for the slide to dry completely.

3.3.9 Staining

Staining highlights the essential features of the tissue and enhances the tissue contrast. Crexyl violet is an effective and reliable stain used for light microscopy sections. It is used to stain the neurons of the brain and spinal cord and demonstrate the Nissl substance in the neurons and cell nuclei (Gurina and Simms, 2020).

3.4.0 Mounting

A dropper is used to apply one or two drops of DPX mountant to the center of the glass slide. To remove any air bubbles, the tissue part is gently placed onto the mountant drop and gently pressed onto the slide. The slide is then covered with a coverslip and tagged with the specimen name (Singh-Bains et al., 2021).

3.4.1 Statistical Analysis

Graph pad prism Software (Version 5.0) was used to analyze the data obtained from the study. The statistical analysis for the respective weights of the animals was done using one-way analysis of variance (ANOVA) using a graph pad prism.

4.0 RESULT

4.1 Body Weight

Table 1 Shows the result of the body weight of experimental animals across all groups. Animals in Group A, the control group, showed an increase in body weight at the end of the experiment. Group B, which received 200mg/kg of *Datura Stramonium* leaf extract, showed an increase in body weight at the end of the experiment. Group C, which received 400mg/kg of *Datura Stramonium* leaf extract, showed an increase in body weight at the end of the experiment. Group D, which received 200mg/kg of *Datura Stramonium* leaf extract with 100mg/kg of Vitamin C, showed an increase in body weight at the end of the experiment. Group E, which received 400mg/kg of *Datura Stramonium* leaf extract and Vitamin C, showed an increase in body weight at the end of the experiment, respectively. Group F, which received 100mg/kg of Vitamin C, showed an increase in body weight at the end of the experiment.

Figure 3 shows an increase in the final body weight of Group A, B, C, D, E and

Table 2: Shows the result of the p-value data obtained from comparison of Group A body weight to other Groups. The level of significance is $P< 0.05$. All values less than 0.05 are statistically significant (*)

TABLE 3 and FIGURE 3 Show the result of the initial versus final weight of experimental animals across all groups. All animals showed increased body weight at the end of the experiment. FIGURE 3: Showed the result of the comparison of the initial and final weight of the experimental animals in group D and F. Group D showed an increase in body weight after receiving 200mg/kg of *Datura Stramonium* leaf extract and 100mg/kg of Vitamin C. Animals in group F showed an increase in body weight.

FIGURE 4: Showed the result of the comparison of the initial and final weight of the experimental animals in groups E and F. Group E showed an increase in body weight after receiving 400mg/kg of *Datura Stramonium* leaf extract and 100mg/kg of Vitamin C. Animals in group F also increase in body weight.

TABLE 4 Shows the weight of the organ (brain) of the experimental animal in each group

Table 1 Shows data analysis results of body weight changes of experimental animals. Data are presented as the (Mean ± S.E.M).

GROUPS	INITIAL WEIGHTS (g)	WEIGHT AFTER WEEK 1(g)	FINAL WEIGHTS(g)
A	100.4 ± 5.767	107.6 ± 7.646	123.6 ± 8.750
B	93.60 ± 5.144	116.6 ± 6.377	129.8 ± 7.664
C	91.40 ± 4.226	115.8 ± 6.799	121.8 ± 9.040
D	96.20 ± 6.793	127.0 ± 7.000	152.5 ± 4.500
E	99.00 ± 4.690	123.0 ± 3.440	141.8 ± 2.839
F	95.40 ± 4.567	106.5 ± 9.921	129.5 ± 10.690

Level of significance is P< 0.05. All values less than 0.05 are statistically significance (*) While all values greater than 0.05 are not statistically significance. All values are expressed in Mean ± S.E.M.

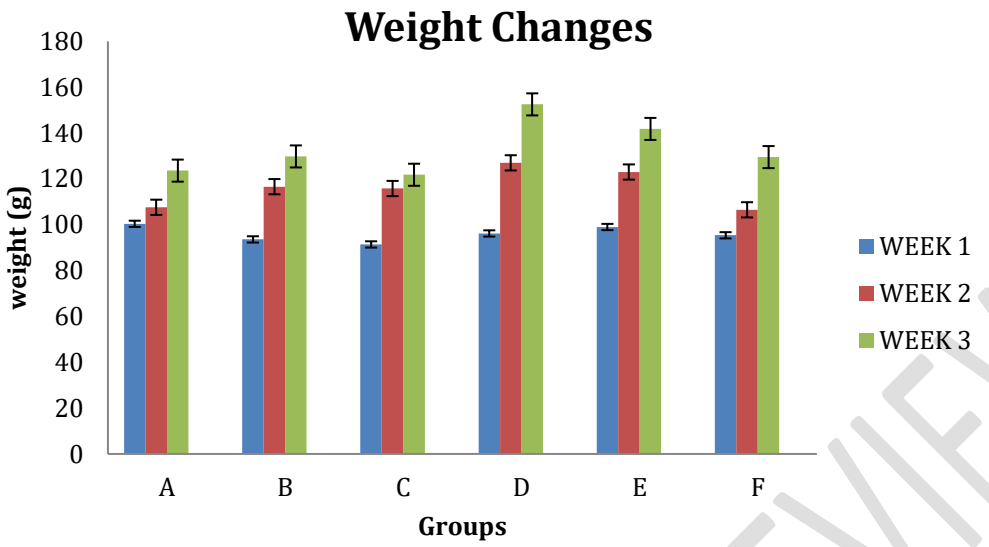


Fig 3 Bar chart showing analysis of Mean ± S.E.M of body weight of Groups A, B, C, D, E, and F across Week 1 to Week 3. Values are represented in Mean ± S.E.M

Table 2 shows the p-value data obtained from comparing the body weights of the groups.

GROUPS	P-Value
A vs B	0.8351
A vs C	0.9438
A vs D	0.4520
A vs E	0.4906
A vs F	0.9959

Significance P: ≤ 0.05. Values greater than 0.05 are considered insignificant, while values less than 0.05 are considered significant. So, all the values obtained for the p-value are insignificant.

Table 3 Shows the initial and final weights of the experimental animals. Values are expressed in Mean ± S.E.M.

GROUPS	INITIAL WEIGHT	FINAL WEIGHT
A	100.4 ± 5.767	123.6 ± 8.750
B	93.60 ± 5.144	129.8 ± 7.664
C	91.40 ± 4.226	121.8 ± 9.040
D	96.20 ± 6.793	152.5 ± 4.500
E	99.00 ± 4.690	141.8 ± 2.839
F	95.40 ± 4.567	129.5 ± 10.690

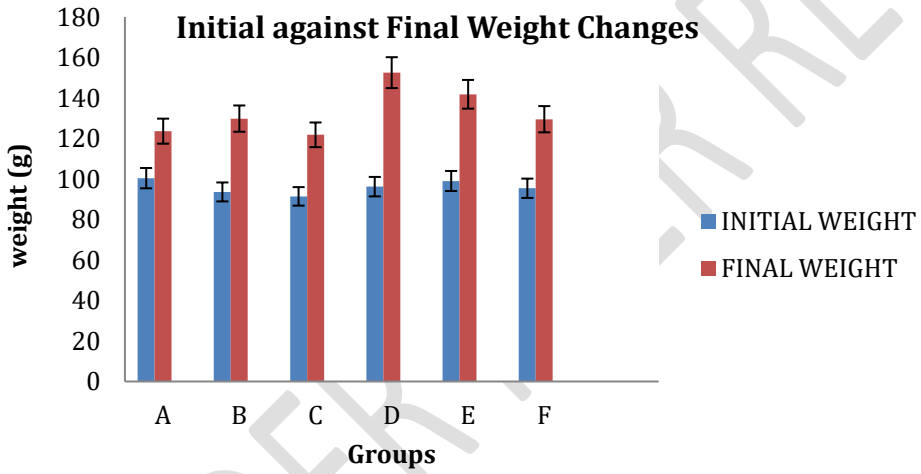


Fig 4 shows bar charts representing data analysis results of experimental animals' initial and final body weight.

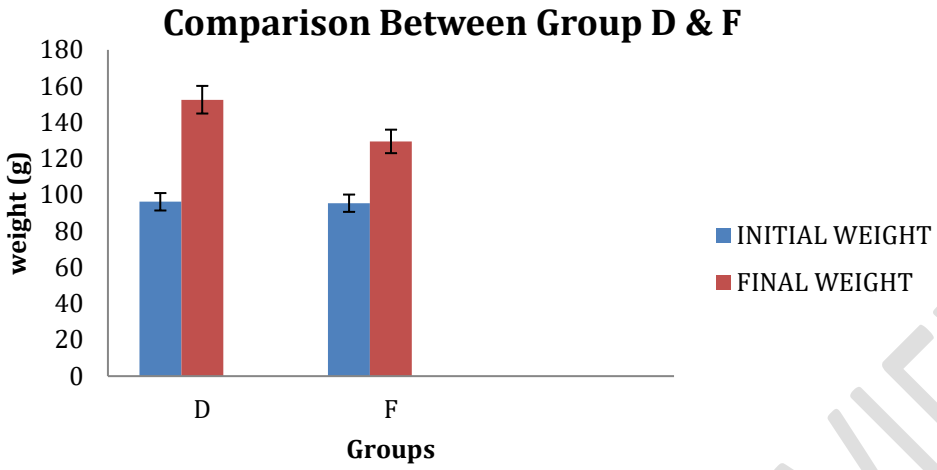


Fig 5 Bar chart showing a comparison of group D against F

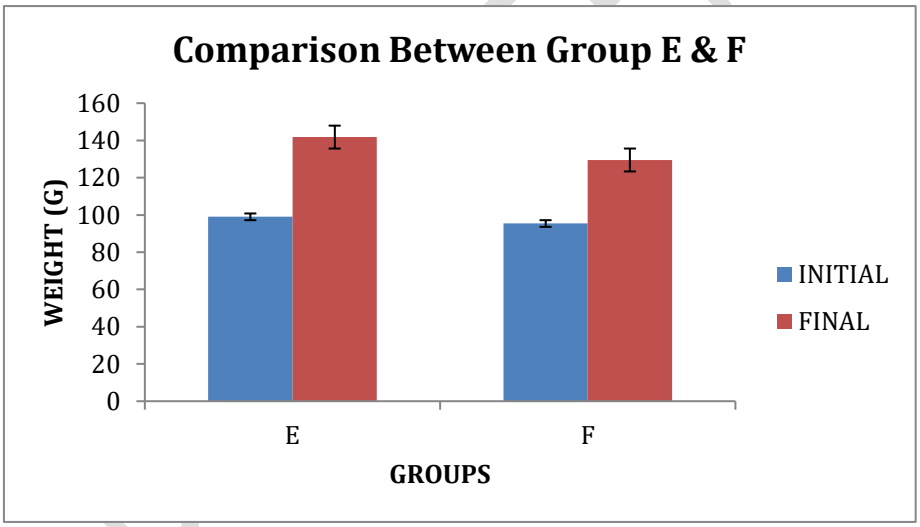


Fig 6 Bar chart showing a comparison of group E against F

ORGAN WEIGHT (THE BRAIN)

Table 4 reveals the mean organ weight of the animals in each group.

Data are presented as mean ± standard error of the mean (Mean ± SEM)

GROUPS	WEIGHT (g)
A	1.340 ± 0.05099
B	1.340 ± 0.04000
C	1.200 ± 0.04082
D	1.500 ± 0.2000
E	1.500 ± 0.1155
F	1.450 ± 0.1756

Significance P: ≤ 0.05. Values greater than 0.05 are considered insignificant, while values less than 0.05 are considered significant in the body weight of the experimental animal.

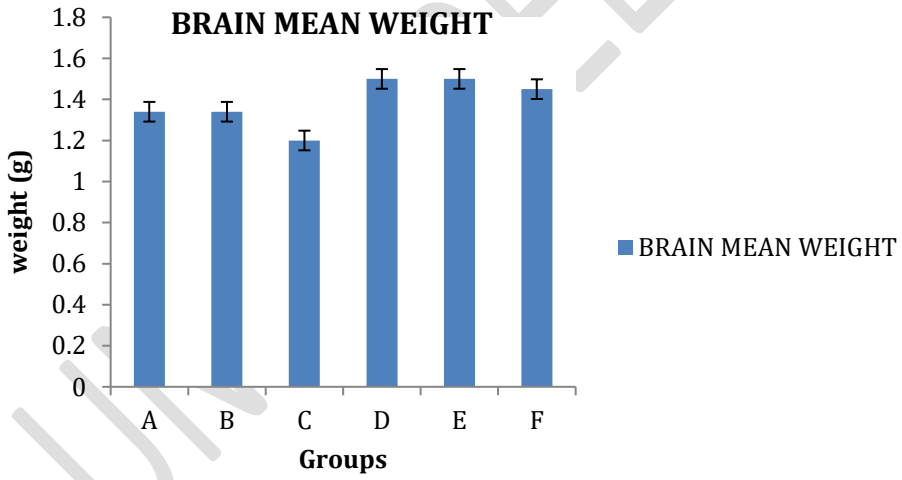


Fig 7 Bar chart showing Mean ± S.E.M of brain weight

GROUPS	Relative Weight Index

A	1.100 ± 0.06607
B	1.045 ± 0.05755
C	1.004 ± 0.08666
D	0.9806 ± 0.1022
E	1.044 ± 0.1099
F	1.131 ± 0.1293

Table 5 shows the relative weight index of the organ (brain) of the experimental animal in each group. Data are presented as mean ± standard error of the mean (mean ± SEM).

Significance P: ≤ 0.05. Values greater than 0.05 are considered insignificant, while values less than 0.05 are considered significant in the relative weight index of the experimental animal.

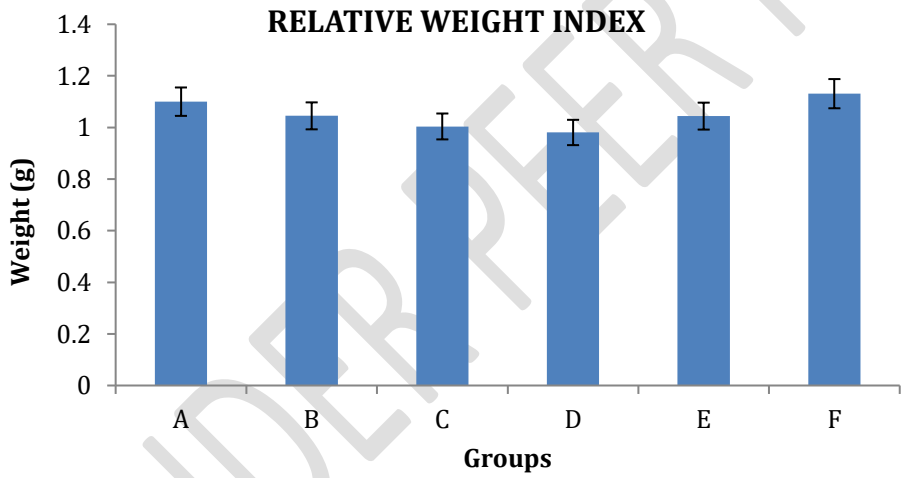


Fig 8 Bar chart showing Relative Weight Index. Values represented in Mean ± S.E.M

Table 6 shows the p-value data obtained from the relative weight index.

GROUPS	P-Value
A vs B	0.5473
A vs C	0.4021

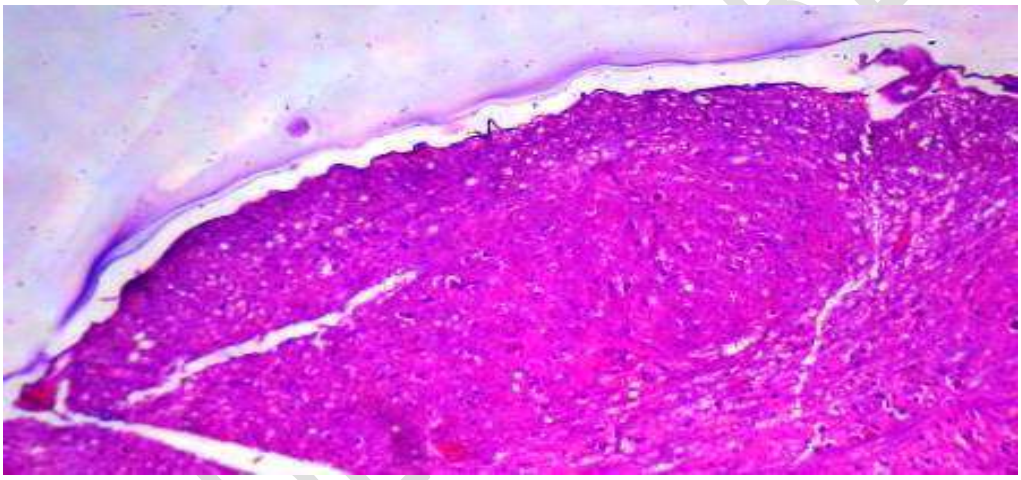
A vs D	0.3780
A vs E	0.6617
A vs F	0.8260

Significance P: ≤ 0.05. Values greater than 0.05 are considered insignificant, while values less than 0.05 are considered significant. So, all the values obtained for the p-value are insignificant.

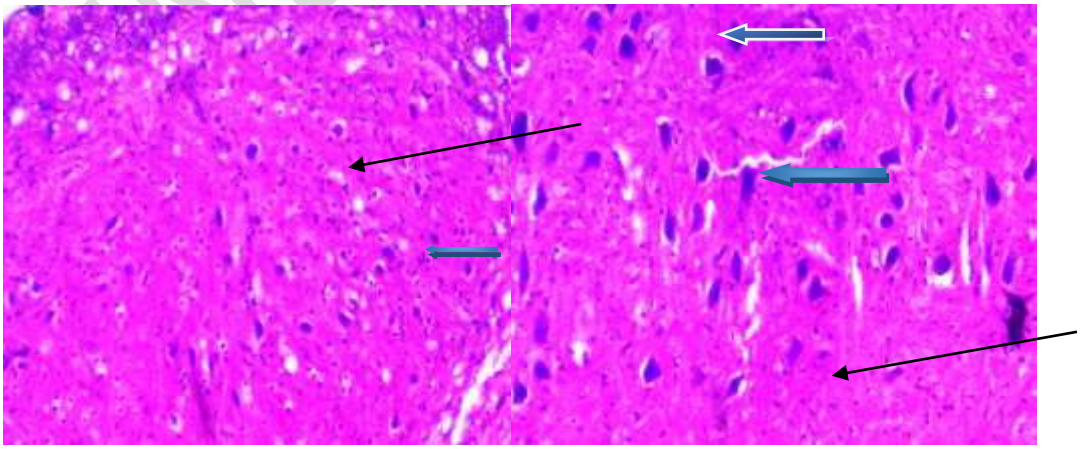
4.2 Histological Findings

PHOTOMICROGRAPH (Hematoxylin and Eosin)

GROUP A



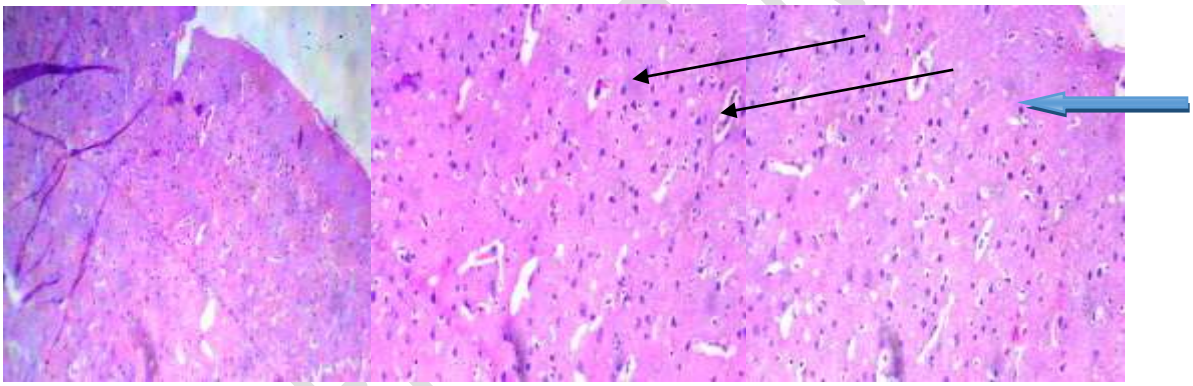
X100



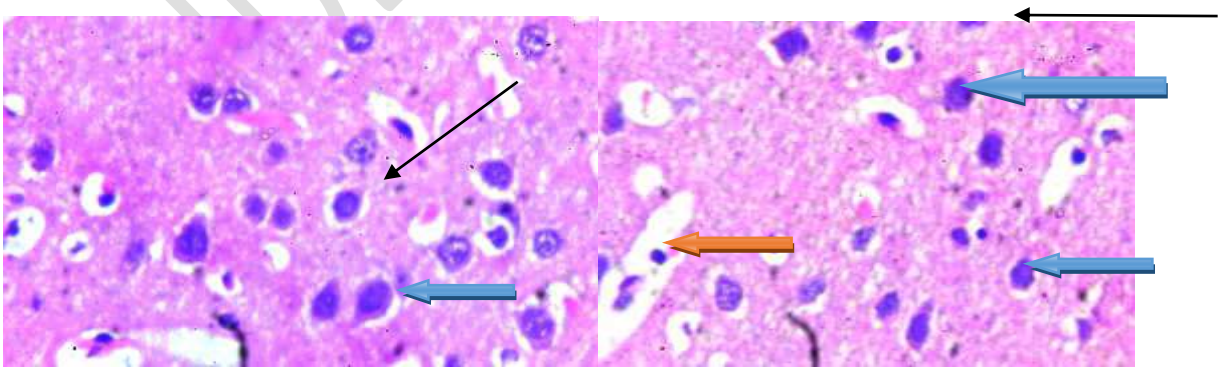
X400

Plate 1& 2: Photomicrograph of a brain section demonstrated by Hematoxylin and Eosin at low magnification and high magnification (X100 and X400) showing the Frontal cortex with normal neuronal cells (blue arrow), the capillaries seen are normal (red arrow), and the stroma also appear normal as well (slender arrow).

GROUP B



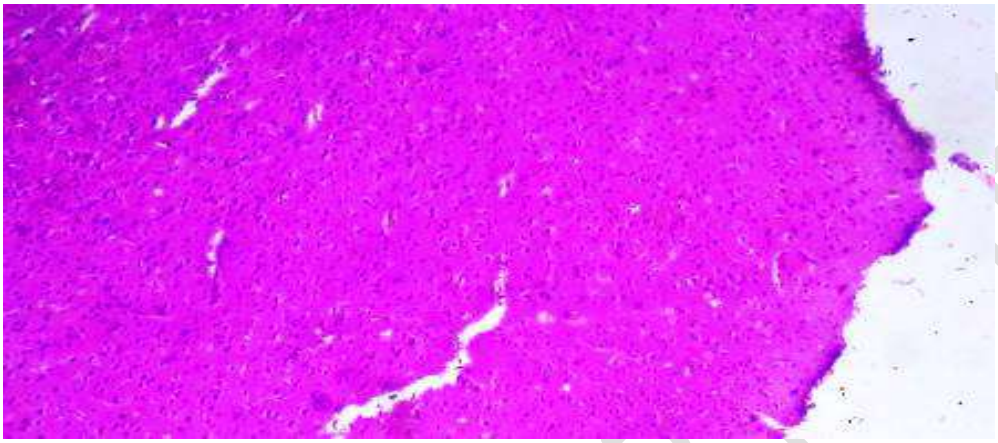
X100



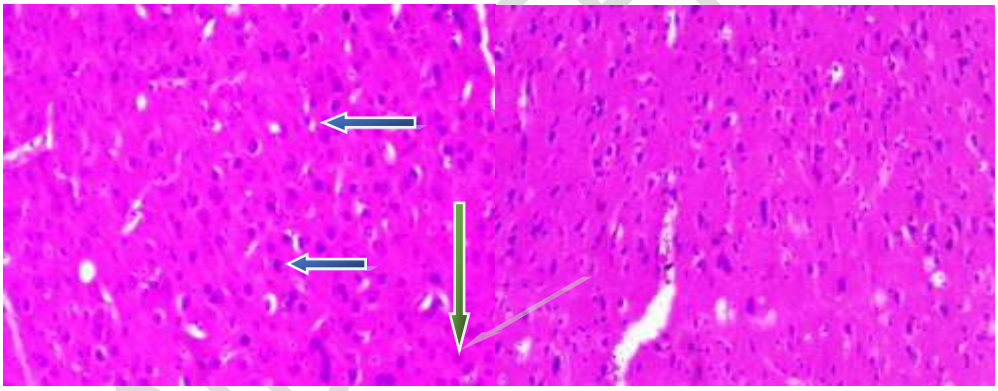
X400

Plate 3&4: Photomicrograph of a brain section demonstrated by Hematoxylin and Eosin at low and high magnification (X100 and X400) showing the Frontal cortex with normal neuronal cells (blue arrow), the capillaries seen are normal (red arrow), and the stroma also appear normal as well (slender arrow).

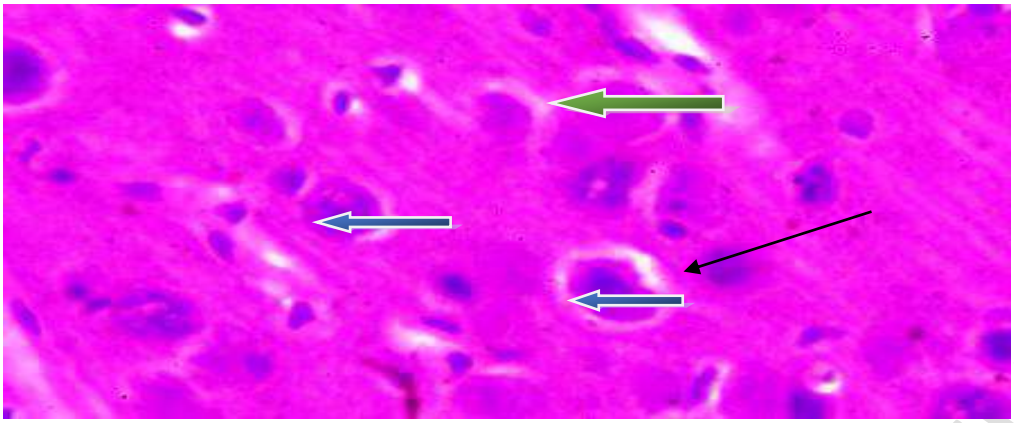
GROUP C



X40



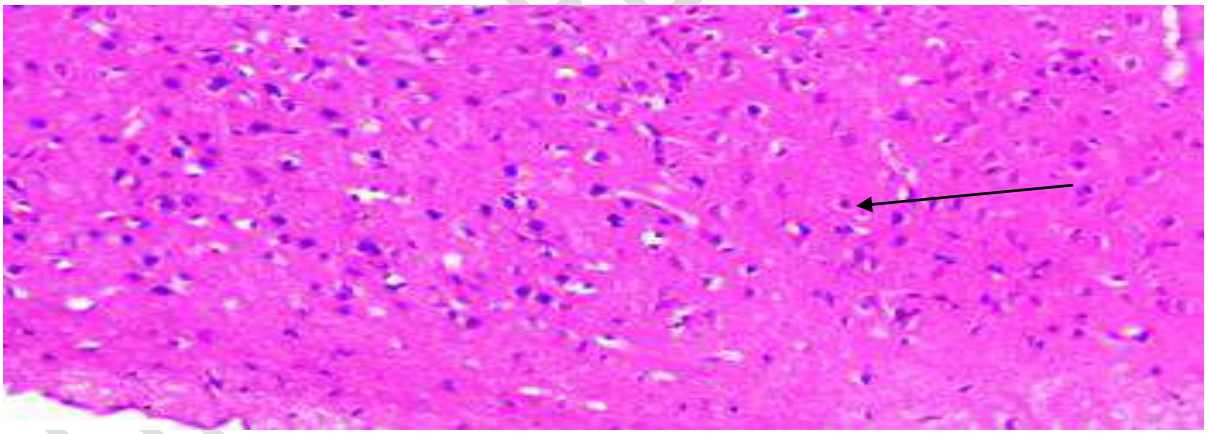
X100



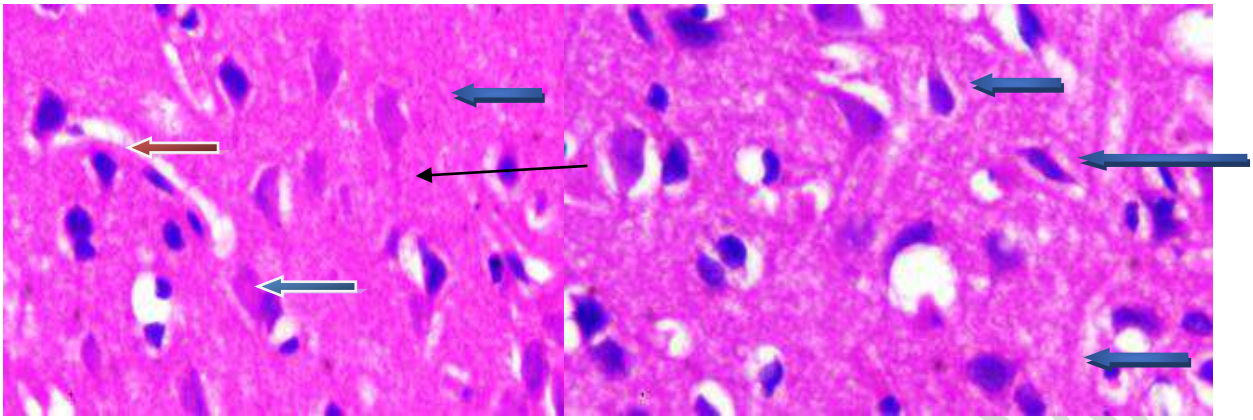
X400

Plate 5, 6and 7: Photomicrograph of a brain section demonstrated by Hematoxylin and Eosin at low and high magnification (X40, X100, and X400) showing the Frontal cortex with some normal neuronal cells (blue arrow) and degenerated neurons with cytochromatolysis (green arrow) and the stroma also appear normal (slender arrow).

GROUP D



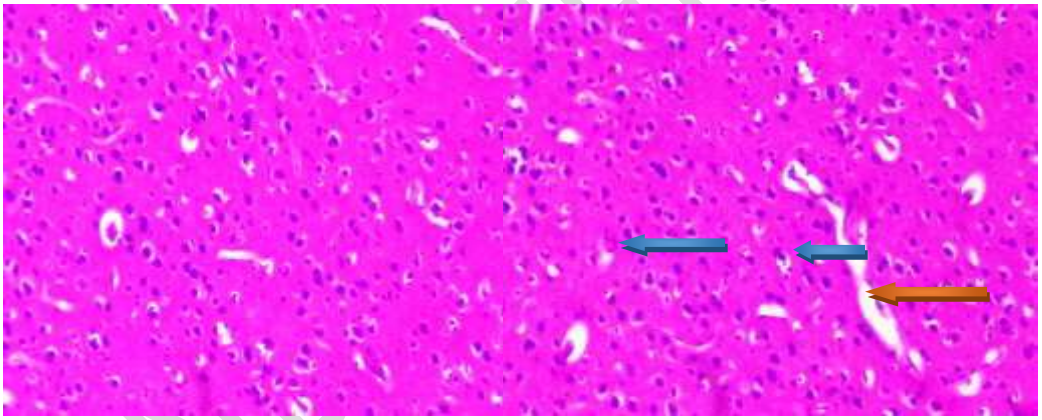
X100



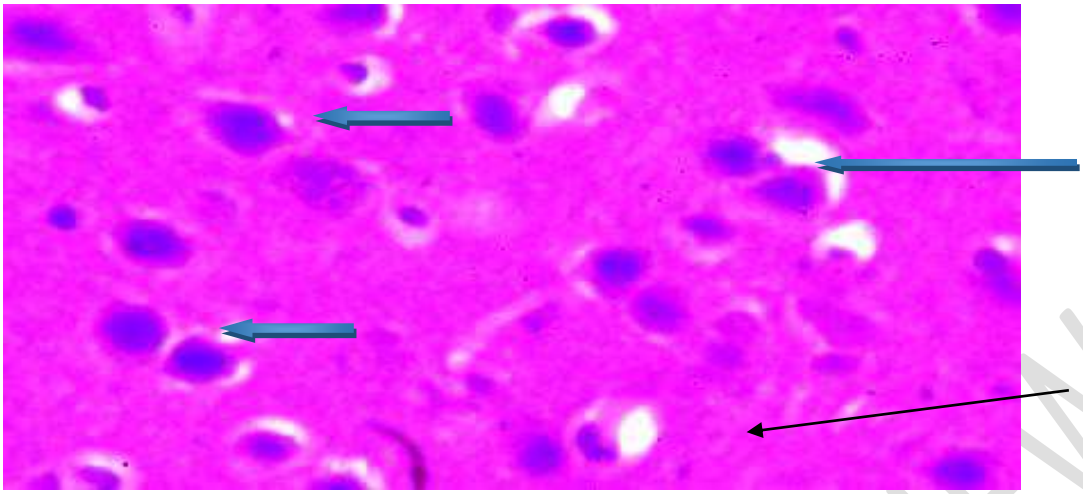
X400

Plate 8&9: Photomicrograph of a brain section demonstrated by Hematoxylin and Eosin at low and high magnification (X100 and X400) showing the Frontal cortex with normal neuronal cells (blue arrow), the capillaries (red arrow) seen are normal, and the stroma also appear normal as well (slender arrow).

GROUP E



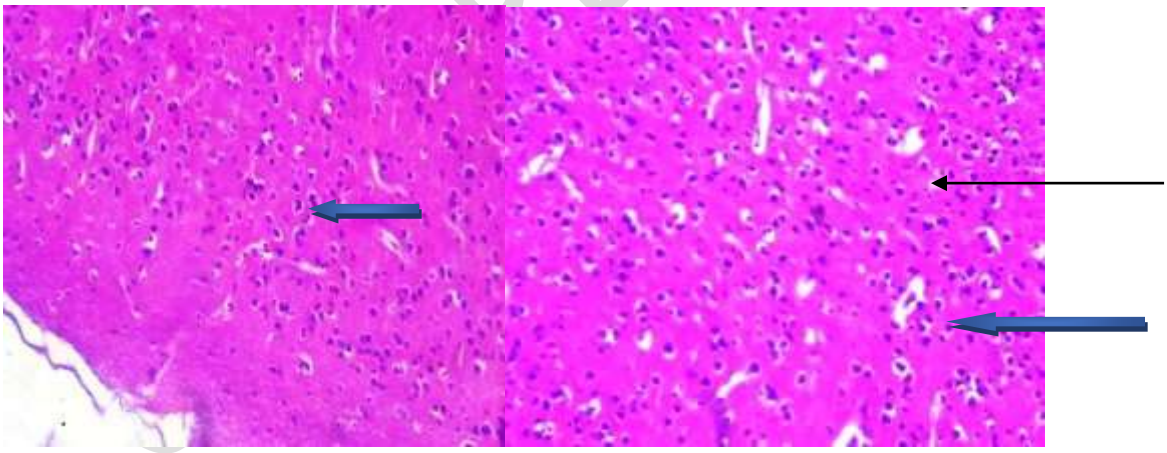
X100



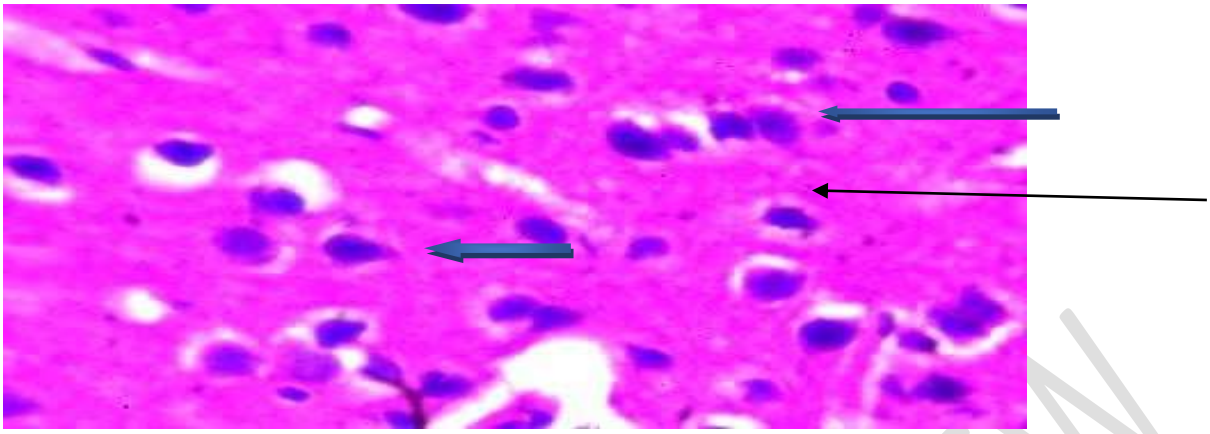
X400

Plate 10&11: Photomicrograph of a brain section demonstrated by Hematoxylin and Eosin at low and high magnification (X100 and X400) showing the Frontal cortex with normal neuronal cells (blue arrow), the capillaries seen are normal (red arrow), and the stroma also appear normal as well (slender arrow).

GROUP F



X100



X400

Plate 12&13: Photomicrograph of a brain section demonstrated by Hematoxylin and Eosin at low and high magnification (X100 and X400) showing the Frontal cortex with normal neuronal cells (blue arrow), the capillaries seen are normal (red arrow), and the stroma also appear normal as well (slender arrow).

5.0 Discussion

Datura stramonium is a widespread annual plant from the Solanaceae family. Consumption of any part of the plant may result in severe anticholinergic reactions that may lead to toxicity and occasionally cause diagnostic difficulties. The major alkaloids hyoscyamine, scopolamine, and several minor tropane alkaloids have been identified in Datura species (Kadam et al., 2018).

The brain is metabolically one of the most active organs in the body and much more susceptible to free radicals and oxidative stress. Oxidative stress has been involved in pathogens and the progression of many neurological disorders. The frontal lobe is responsible for responses relating to memory, emotions, reasoning, judgment, planning, and verbal communication. This lobe is vital to health, so any structural alteration should be avoided (Crossman and Neary, 2018; Yu et al., 2020).

In their study, (Ekanem et al. 2016) found that the administration of *Datura stramonium* leaf extract at a specific concentration induced neurotoxicity in the cerebral cortex, as observed in electron photomicrographs. The statistical analysis shows that all groups of experimental animals gained weight compared to their initial weight.

Experimental animals in groups B and C were administered orally 200mg/kg (low dose) and 400mg/kg (high dose) body weight respectively of ethanoic extract of *Datura stramonium* leaf, which revealed an increase in final mean weight, showing no statistical significance ($p<0.05$), when compared with initial results. This is in support of findings by (Animoku et al., 2019).

Co-administration of 200mg/kg (low dose) body weight of ethanoic extract of *Datura stramonium* leaf and 200mg/ml of vitamin C and Co-administration of 400mg/kg (high dose) body weight of ethanoic extract of *Datura stramonium* leaf and 200mg/ml of vitamin C to Groups D and E respectively shows an increase in their final mean weight but shows no statistical significance ($P<0.05$).

Administration of 200mg/ml of Vitamin C to the experimental animals of group F shows an increase in the final mean weight but shows no statistical significance ($p<0.05$).

Analysis of the relative weight index shows an increase in the relative weight of organs in all groups compared to the control group.

The histological analysis from the study shows that the frontal cortex in all groups (A, B, D, E, and F) had normal neuronal cells and stroma. Group A did not show any significant changes in the histology of the neurons of the frontal cortex. There were no definite patterns of neuro-degeneration recorded in the FC neurons of Group B compared to the control (Group A). This could imply that lower dosage may not produce a clear pattern of neurodegeneration in the frontal cortex; this is in support of (Ekanem *et al.*, 2016).

On the other hand, Group C was found to induce neurotoxicity in the frontal cortex, as observed in photomicrographs (Plate 5, 6, and 7), which showed evidence of neurodegeneration compared to other groups. Group D, E, and F, which received ascorbic acid, did not show any significant changes in the histology of the frontal cortex neurons.

6.0 Recommendation

Precaution should be taken when consuming *Datura stramonium* leaf because it may adversely damage the neurons in the frontal cortex. Further studies should be done to investigate the behavioral, biochemical, and neurochemical alterations in the neurons of the frontal cortex challenged with varying doses of *Datura stramonium* leaf extract.

7.0 Conclusion

Administration of the ethanolic extract of *Datura stramonium* leaf at excessive dosages induced significant histological alterations in the neurons of the frontal cortex in Wistar rats, including neuronal cell death and axonal atrophy.

CONSENT

It is not applicable

ETHICAL APPROVAL

Animal Ethics Committee approval has been collected and preserved by the author(s).

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