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PROTECTIVE EFFECTS OF VITAMIN C AND E ON AMYGDALA OF METHAMPHETAMINE INDUCED BRAIN DISORDER ON ADULT MALE WISTAR RATS

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ABSTRACTS

This study examined the protective effects of vitamin (vit.) C and E on methamphetamine (meth) induced brain disorder in adult male Wistar rats. 56 adult male Wistar rats were randomly divided into 7 groups of 8 animals each, for a period of 14 days. Group A received normal saline and showed normal neurological features at tissue level, group B received meth and showed impairment on both histoarchitectural and immunohistochemical level, group C received vit. C and showed normal neurological features at tissue level, group D received vit. E and showed normal neurological features at tissue level, group E received meth and vit. C and showed reversal of meth induced neuronal changes, group F received meth and vit. E and also showed reversal of meth induced neuronal changes, group G received meth, vit. C and E and showed synergistic improvement in reversal of meth induced neuronal changes Vit. C treatment reduced malondialdehyde (MDA) and tumour necrosis factor alpha (TNF-α) levels, improved histoarchitectural arrangement of neurons in hematoxyline and eosin (H and E) and cresyl violet (CSV) stain, and increased superoxide dismutase (SOD), glutathione (GSH-Px) and B cell lymphoma-2 (BCL-2) levels, vit. E treatment showed similar effects with vit. C with much improvement, while both vit. C and E combined together produced synergistic effect.

In conclusion, this study demonstrated that treatment with meth resulted in weight loss, though not statistically significant and vit. C and E treatment improved weight gain. Also, treatment with meth resulted in a decline in total brain weight and vit. C and E improved the

total brain weight. Treatment with meth also resulted in increased MDA activities in the amygdala and a decline in SOD and GSH levels which were ameliorated by treatment with vit. C and E. Further, treatment with meth resulted in decreased cell count on H and E and CSV stains and increased count on TNF-α stain, though not statistically significant. Treatment with vit. C and E offered some protection. Treatment with meth showed significant decrease in BCL-2 level which were improved by vit. C and E.

KEYWORDS: Metheamphetamine, Vitamin C and E, Amygdala.

INTRODUCTION

Meth use among young people is of significant social, economic and public health concern to affected communities and policy makers (Marshall and Werb, 2010). Substance abuse has been implicated as a major cause of mental health disorder with 52.3% mentally challenged respondent, depending on various addictive used (Stanley and Chinwe, 2022). A 2018 United Nations Office on Drugs and Crime (UNODC, 2018) report estimated that 89, 000 Nigerians were taking meth without effective health intervention to mitigate its degenerative effect on the brain. The recent abuse of meth in our immediate communities are predominantly among young adults, a study by National Institute on Drug Abuse (NIDA, 2007) suggests crystal meth use in young adults higher than previously reported. It is a powerful stimulant of abuse with potent addictive and neurotoxic properties (Moszczynska, 2016). More than 2.5 decades ago, meth-induced damage to dopaminergic neurons was described (Riddle, Fleckenstein and Hanson, 2006; Shrestha *et al.* 2022).

Meth, also known as “ice” or “crystal”, is one of the most widely abused illicit amphetamine-type stimulants (Courtney and Ray, 2014; Jones, Compton and Mustaquim, 2020). Addiction to Meth is regarded as an international public health problem with over 27 million users worldwide (0.5% of the global population), since it is easy to manufacture and is cheaply available (Moszczynska, 2021). The drug increases levels of certain neurotransmitters in the brain, including dopamine, norepinephrine, and serotonin which plays key roles in regulating mood, motivation, and pleasure (Prakash *et al*. 2017).

Meth is taken orally, intravenously, or insufflated (Boyer, Seifert, Hernon, and Burns, 2019). After ingestion, meth is rapidly absorbed into the bloodstream, and it crosses the blood-brain barrier, allowing it to reach the brain quickly (Boyer *et al.* 2019). Once in the brain, meth increases the release of dopamine from nerve terminals in the brain's reward center.

Dopamine release leads to a surge of intense pleasure and euphoria, which is a significant contributor to the drug's addictive potential (Prakash *et al.* 2017). Meth's primary ingredients, ephedrine and pseudoephedrine, come from over-the-counter cold medications and weight loss products (Yu, Wang, and Cheng, 2018).

Clinical manifestation of meth addiction depends on the dose and duration of use (Paulus and Stewart, 2020).

Consistent associations were observed between meth use and several mental health outcomes, including depression, suicidal ideation and psychosis (Marshall and Werb, 2010). Negative side effects of meth abuse mostly include increased anxiety, tremors and hallucinations (Moszczynska, 2016). Moreover, long-term use of meth causes paranoid psychosis and cognitive impairment such as memory loss (Paulus and Stewart, 2020).

Previous studies have shown that meth abuse can lead to extensive damage to the brain, highlighting the potential need for interventions to reduce this damage (Yu, Zhu, Shen, Bai and Di, 2015).

Meth use has been shown to cause oxidative stress, inflammation, and excitotoxicity, which promote the death of neurons in the brain (Kim, Yun, and Park, 2020; Yang *et al.* 2018). These processes affects the amygdala leading emotional impairments (Mizoguchi and Yamada, 2019; Prakash *et al*. 2017).

Oxidative stress, an imbalance between the production of reactive oxygen species and the body's antioxidant defenses, has been implicated in the pathogenesis of neurodegenerative diseases and cognitive decline (Poljsak, Šuput and Milisav, 2013), psychiatric disorders and emotional dysregulation (Salim, 2014; Taschetto *et al.* 2017).

Despite the abuse of meth among youths and its attendant health effects in the society, compounds to mitigate these effects are not well-characterized (Acheson *et al.* 2023).

There is limited literature on evidence-based pharmacological treatment approaches for meth withdrawal (Rawson, 2013).

Antidepressants and anxiolytics may be used to ameliorate depressive and anxiety symptoms, although research suggests only limited benefits of antidepressants in reducing withdrawal symptoms (Shoptaw, Kao, Heinzerling and Ling, 2009).

Neuroleptics may be used to treat meth-induced psychotic symptoms in the context of intoxication or recent use (Shoptaw *et al.* 2009).

Leelahanaj, Kongsakon and Netrakom (2005), in a study demonstrated the equivalent efficacy of olanzapine, an atypical neuroleptic, and haloperidol, a typical neuroleptic, in improving psychotic symptoms related to amphetamine use but does not completely alleviate the symptoms.

Treatments for meth use disorder are currently limited to behavioral and synthetic drug therapies that are generally unsuccessful. Consequently, the National Institute on Drug Abuse has declared research to identify medications to treat substance use disorder a priority (Kostov, Angelone, Gutowski, and Koustova, 2023).

Some researchers have proposed the use of anti-meth antibodies in the bloodstream for the treatment of meth abuse. However, their study only presented preliminary results, and the animal numbers were not sufficient to satisfy statistical criteria (Chen, Wu, Tsai and Chen, 2013).

There are reports that neurodegenerative diseases such as Alzheimer's, Parkinson, multiple sclerosis and other forms of dementia related diseases may be preventable if there is prior neuronal protection using herbs and other vitamin rich food types (Barnard *et al.* 2014; Liu *et al.* 2020).

The cell has several ways to alleviate the effects of oxidative stress either by repairing the damage damaged nucleotides and lipid peroxidation by products) or by directly reducing the pro-oxidative state via enzymatic and non-enzymatic antioxidants (Sharm, Gupta and Prabhakar, 2019).

Non-enzymatic (Vit. E and C, urate, melatonin etc) and enzymatic -SOD, GSH-Px and Catalase (Ahamad, Ansari, Negi and Khan, 2019).

Antioxidants have been shown to scavenge free radicals and ROS (Ahmadinejad, Møller, Hashemzadeh-Chaleshtori, Bidkhori and Jami, 2017).

Vit. C**,** also known as ascorbic acid, is a water-soluble vit. that has several important functions in the body (Agwu, Ezihe and Kaigama, 2023). It is an essential nutrient that cannot be synthesized by the human body and therefore must be obtained through dietary sources or supplements (Li and Schellhorn, 2007). It aids in enzyme activation, oxidative stress reduction and immune system enhancement (Olaniran *et al.* 2023). It is widely present in biological systems as a naturally occurring free radical scavenger (Chambial, Dwivedi, Shukla, John and Sharma, 2013; Pehlivan, 2017).

This vit.C appears as an ascorbate monovalent anion at physiological pH (Corti, Casini and Pompella, 2010; Naidu, 2003). It acts as a potent antioxidant that helps to protect cells from oxidative stress and damage. It also plays a critical role in the synthesis of collagen, the regeneration of other antioxidants in the body (Chambial *et al.* 2013). It exerts neuromodulatory functions and scavenges reactive oxygen species generated during synaptic activity and neuronal metabolism (Moretti, Fraga, and Rodrigues, 2017).

Intracellular Vit. C helps maintain integrity and function of several processes in the central nervous system (CNS), including neuronal maturation and differentiation, myelin formation, synthesis of catecholamine, modulation of neurotransmission and antioxidant protection (Kocot, Luchowska-Kocot, Kiełczykowska, Musik and Kurzepa, 2017).

Vitamins and different phytochemicals are important epigenetic modifiers that prevent neurodegeneration (Carrera, Martínez, and Cacabelos, 2019). Studies have found that vit. C can reduce the neurodegeneration associated with traumatic brain injury, Parkinson's disease, and Alzheimer's disease (Kocot *et al*. 2017).

Similarly, vit. E has first line of defense against the peroxidation of lipids (Jomova *et al*. 2023). It has anti-inflammatory and neuroprotective effects, with research studies finding potential for its use against multiple sclerosis, stroke, and neurodegenerative diseases (La Torre *et al*. 2021). Ungurianu, Zanfirescu, Nițulescu, and Margină (2021), inferred that vit. E can exert protective effects against oxidative stress-induced neurodegenerative diseases. Oxidative stress, an imbalance between the production of reactive oxygen species and the body's antioxidant defenses, has been implicated in the pathogenesis of neurodegenerative

diseases and cognitive decline (Poljsak *et al.* 2013), psychiatric disorders and emotional dysregulation (Michels *et al*. 2018). One of the primary mechanisms through which vit. E exerts its neuroprotective effects is by scavenging free radicals and reducing oxidative damage to neuronal structures (Crouzin *et al.* 2010). Vit. E's antioxidant properties enable it to neutralize reactive oxygen species and protect the neurons from oxidative stress-induced injury (Gueroui and Kechrid, 2016).

Vit. E can modulate neurotransmitter systems in the brain, regulating their release and metabolism thereby influencing cognitive functions, emotional and fear-related behaviors (Kumar, Rinwa, Kaur and Machawal, 2013). It has been shown that vit. E supplementation can enhance the levels of key neurotransmitters, such as acetylcholine, serotonin, dopamine and glutamate, in the amygdala which is essential for and cognitive processes, emotional processing and fear conditioning (Adalier and Parker, 2016; Ulatowski and Manor, 2015).

These findings suggests that vit. E may play a crucial role in regulating the neurochemical balance.

In addition to its antioxidant and neurotransmitter-modulating actions, vit. E has been found to maintain the structural integrity of the cerebral cortex (Ulatowski *et al*. 2014). Animal studies have revealed that vit. E administration can protect against age-related cortical atrophy, stress-induced neuronal damage, preserve dendritic morphology, and maintain synaptic connectivity (Liao *et al*. 2022; Rizvi *et al*. 2014).

The amygdala is a small almond-shaped structure located deep within the medial temporal lobes (Mohammadi, Haghir, Fazel and Vafaei, 2013). It is located on both sides of the brain and is a part of the limbic system which is involved in processing and regulating emotions, memory, and motivation (Šimić *et al*. 2021). This brain region is involved in emotions such as fear, anxiety, and stress (Forster, Novick, Scholl and Watt, 2012; LeDoux, 2007). When the amygdala detects a potential threat, it initiates the stress response by activating the hypothalamus and the autonomic nervous system, this leads to the release of stress hormones like adrenaline and cortisol, preparing the body for a fight-or-flight response (Adolphs and Anderson, 2018; Pessoa, 2020).

It is composed of several sub nuclei and interconnected networks that allow it to receive and send signals to other brain regions involved in emotion, memory, and decision-making (Gongora *et al.* 2019).

Exposure of amygdala to meth has been reported to decrease the number and density of neurons in the amygdala (Zuloaga, Johnson, Weber and Raber, 2016). Additionally, meth has been found to induce neuronal damage, including dendritic atrophy and loss of synaptic connections in the amygdala (Shrestha *et al.* 2022).

Meth use also affects neurotransmitter systems within the amygdala. For example, studies have demonstrated that meth exposure caused changes in dopamine and serotonin levels in the amygdala, which have been linked to alterations in reward, motivation, and emotional processing (Robinson, Warlow and Berridge, 2014; Walker and Nestler, 2018). Moreover, meth-induced dysregulation of glutaminergic signaling in the amygdala has been implicated in the development of drug craving and relapse (Li, Zeric, Kambhampati, Bossert and Shaham, 2015). Meth can over activate the amygdala, leading to an increase in anxiety disorder and agitation (Harro, 2015; Kawano *et al.* 2018).

Individuals with a history of meth use have been shown to exhibit emotional dysregulation, increased impulsivity, and heightened sensitivity to drug-associated cues, which are related to amygdala dysfunction (Dean, Kohno, Hellemann, and London, 2014).

MATERIALS AND METHODS

Location and Duration of the study

This study will be carried out in the Department of Anatomy, Faculty of Basic Science, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. The rats will be acclimatized for a period of two weeks, after which the test substances will be administered for 14days. Hence, the entire experiment will last for about four weeks.

Materials used for the study

The materials that will be used includes; meth, vit. C and E, Iron cages with iron nettings, Saw dust (litter), Animal feed (Grower and Finisher mash) and water, Laboratory coat and gloves, One stopwatch, One digital timer, a digital video recorder, Clean caps of 15 ml conical tubes, distilled water, Dissecting set and dissecting board, T shaped wooden structure,

suspension box, Tape, cleaning chemicals (70% ethanol), laboratory-bred plus maze-naïve rats, paper towels, elevated plus maze apparatus, lux meter, measuring cylinder and plastic specimen bottle, weighing balance, water bath, spray bottle, sample bottle, 10% Neutral Buffered Formalin, Cotton, anaesthesia (chloroform), Graded alcohol (50%, 70%, 95% and absolute alcohol), glass slide and slide rack, hot plate, xylene, paraffin wax, embedding plate and pot, Deepex (DPX) mountant, haematoxylin and eosin, cresyl violet, cover slip, light microscope, Orbit shaker, diamond pencil, rotatory microtome, ethanol for tissue processing, micro pipette, specimen labels and bottles, trays, paraffin dispenser, cassette and slide storage, pipette tips (various sizes), Knife sharpener, Notebook and biro, analytical weighing balance, syringes, canula, hand gloves, water.

Materials that will be used for histology includes; 10% formal saline, Xylene, Absolute alcohol, Paraffin Wax, Egg albumin, Hematoxlyn, 1% acid alcohol, Eosin, D.P.X, Slide and Cover slip, Hot air Oven, Microtome, Hot plate, Water bath, Wax dispenser, Microscope, Photomicrograph and Computer.

Collection of material

Meth hydrochloride will be purchased under Nigeria government specified permit number, kept under a specific license number and usage logged as prescribed by national legislation.

The substance will be kept in a cool dry place, dissolved in distilled warm water freshly for oral use daily.

Procurement of vit. C and E- The vit. C and E to be used in this study will be obtained from a reliable pharmaceutical shop in Nnewi, and their quality and purity will be assessed before use to be sure it meets the standard required for the experiment.

It will be stored according to the manufacturer's instructions, in a cool, dry place away from sunlight and other sources of heat, in order to maintain their stability and potency.

The feed that will be used is normal growers mesh produce.

Ethical approval

Ethical approval will be obtained from the animal research ethics committee, Nnamdi Azikiwe University, Awka.

Drug preparation

Meth (purity>98%), will be freshly dissolved in warm distilled water before usage and administered orally at a dosage of 5mg/kg (Ares-Santos *et al*. 2012) twice daily for 14 days. Animals in the control group received saline solution only with the same dosage.

Preparation of vit. C and E supplements will be done daily by dissolving them in distilled water to achieve the desired doses for oral administration. Vit. E will first be dissolved in tween 80 before addition of distilled water. The dosage to be used for both is 200mg/kg (Kalender *et al*. 2010).

Handling will be done under aseptic conditions to prevent contamination.

The supplements will be measured accurately using calibrated syringes and administered orally to the rats in the treatment groups.

Acute Toxicity Study of Vitamin C and E, and Methamphetamine

The median lethal dose (LD50) of Vit. C, vit. E, and meth will be carried out in the department of Physiology, Faculty of Basic Medical Science, Nnamdi Azikiwe University, Nnewi Campus, Nnewi. This will be determined using a method of Dietrich Lorke (1983) with modification. In this study, 13 rats will be used. The substances (drugs) will be via oral route for vit. C and E, while meth will be through intra-peritoneal route and it will be carried out in two phases.

Phase I

A total of nine (9) rats were employed for the study and three rats will be allocated for each group.

Group 1 received 10mg/kg per rat

Group 2 received 100mg/kg per rat

Group 3 received 1000mg/kg per rat

The animals will be monitored for 24hrs for morbidity and mortality. The rats remained normal after 24 hours of observation in phase 1. Then, the study proceeded to the second phase will be four rats will be employed for the study comprising one rat per group.

Phase II

Group 1 received 1200mg/kg per rat

Group 2 received 1600mg/kg per rat

Group 3 received 2900mg/kg per rat

Group 4 received 5000mg/kg per rat

The animal will be monitored for another 24hrs for morbidity and mortality.

Experimental Animals and Design

The research will be done with fifty six (56) adult male albino Wistar rats weighing between 180g-240g. The rats will be procured from a local farm at Nnewi (a reputable supplier that follows ethical guidelines for animal treatment). They will be housed using 7 perspex cages, randomly grouped into 8 rats in each cage (A-G) in a specific pathogen-free environment under standard condition of illumination, temperature and humidity. Each cage is expected to have wire gauze top for cross ventilation. The cages will be cleaned regularly to minimize health risks and to prevent infections.

The animals will be kept at the animal house of Anatomy Department, Faculty of Basic Science, College of Health Science, Nnamdi Azikiwe University, Nnewi Campus, under a controlled room temperature of 25-28°C, relative humidity of about 60-80% and photo-periodicity of 12h day / 12h night.

The rats will be acclimatized to the laboratory environment for two weeks before initiating the study. They will be fed with water and feed (grower mash) *ad libitum* from Agro feed mill Nigeria Ltd. All the animals will be treated in guidance with the approval of ethical committee in the Faculty of Basic Medical Sciences, College of Health Science, Nnamdi Azikiwe University, Nnewi Campus, in compliance with the "National Institutes of Health Guide for the Care and Use of Laboratory Animals" (NIH, 2011).

Animal Groupings and Administration

A total of fifty six (56) adult male Wistar rats will be procured for this study, they will be weighed and randomly divided into 7 groups of eight animals each. The groups will be identified as A, B, C, D, E, F and G.

Group A will receive feed and water only. Meth (5mg/kg) will be given orally to rats in groups B, E-G twice daily (6am, 6pm) for 14 consecutive days to induce brain disorder. Rats in groups C will receive Vit. C 200mg/kg daily (6 am), rats in groups D will receive Vit. E 200mg/kg daily (6 am), rats in group E will receive meth + vit. C, rats in group F will receive

meth + vit. E, while rats in group G will receive meth + vit. C and E. All administrations will be done orally using oral-gastric cannula, between for a period of 14 days. The substance will be administered at room temperature with a pH level of 9.2.

Behavioural testing

After 14 days of meth exposure and treatment, all rats will be subjected to behavioral testing, a day after the last exposure.

1. Morris water maze test

Spatial learning and memory will be assessed using the Morris water maze. The procedure will be performed as described by (Joca, Zuloaga, Raber and Siegel, 2014). The apparatus consists of a large circular pool (tank) about 6 ft in diameter and about 3 ft deep. The inside of the tank will be painted white and the outside brown. It will be filled up with tap water at temperature about 25°C under bright lighting. The maze will be divided into four virtual quadrants, a platform of 24 cm high and 10 cm in diameter will be placed at one quadrant of the pool. During training, the platform will be exposed 1 inch above the water. This will teach the rat that there is a platform, and that it is the way to get out of the water. Animals that were unable to locate the platform during training will be led there by the experimenter and allowed to remain on the platform for 3 seconds. Prior to the test session, the platform will be submerged in the quadrant where it was during the training session as the animal will be retrained to locate the submerged platform hidden in one of the four quadrants. Each test session will involve three 60-second trials with an approximately 10 s inter-trial interval. After the animal is trained and ready for the test session, the escape platform will be 1 inch below the water, and the water will be made opaque by adding non-toxic white tempera paint. The opaque and visible training test session will be done across a single day. During the test session, if the animal failed to climb onto the platform within 60 s, the trial will be stopped and the animal will be removed from the water and placed on the platform. The inter-trial interval is 10 s, the animal will remain on the platform during this inter-trial interval before beginning the new trial. At the end of the three trials, the animal will be removed and placed under a lamp for warmth. The inter-session interval will be 1 h. A stop watch and camera will be used to manually record swimming patterns and to measure swim speed and escape latency.

One probe trial will be done after the test session on the same day, in which the platform is removed from the pool. The probe trial will be performed to verify with great precision

whether the animal understands the location of the platform and the strategy that the animal follows when it discovers that the platform is not there. The number of times the animal crossed the position of the removed platform will be counted as probe trial value and also the percentile quadrant time will be taken as the amount of time the animal spent in individual quadrant during the probe trial, searching for the removed platform.

2. Elevated plus maze

The Elevated Plus Maze (EPM) test will be used to assess anxiety-related behavior in the animals for CNS disorders. The EPM apparatus consists of a "+" shaped maze elevated above the floor with two oppositely positioned closed arms, two oppositely positioned open arms, and a center area. This is a five mutes test which will allow the subjects to freely explore the maze, their behavior is recorded by means of a video camera mounted above the maze and analyzed using a video tracking system. The preference for being in open arms over closed arms (Expressed as time spent in the open arms) is calculated to measure anxiety-like behavior.

Procedure

1. The maze will be assembled in an isolated room away from any extraneous interference of noises, scents or movement.
2. There will not be excessive noise or movement during the entire trial, also no wearing perfumes, colognes or any product with a strong smell, since it could act as anxiogenic stimulus for rats.
3. Illumination in the room will be measured with the aid of a lux meter, kept constant and controlled according to the analysis that is to be performed. Given that low-intensity luminosity reduces open arm avoidance (Nin *et al.* 2012), to analyze an anxiogenic effect, low-intensity lighting (5-30 Lux) should be preferred, whereas an anxiolytic effect should be analyzed under higher intensity lighting (200-400 Lux or more).
4. After these experimental conditions are adjusted to a standard, the animals will be brought into the experiment room, where they will be left in their home cages for 45 to 60 min in order to recover from the stress of being moved.
5. The maze will be cleaned with 70% ethanol before starting the test in order to remove any dirt or smells accumulated on the apparatus.
6. The video camera will be turned on and the first rat placed in the center square of the maze facing one of the open arms, preferably the one opposite to the experimenter.

7. Experimenter stands as far away as possible from the maze and out of sight of the test animal, outside of the room if necessary. No unnecessary movement or sounds.
8. After 5 min of free exploration, the rat will be moved out of the maze and back into its home cage.
9. All the urine and fecal boli will be removed and the maze cleaned entirely with 70% ethanol to remove any residual smell from the first rat. Afterwards, the next rat will be submitted to the test.
10. The experimenter will repeat steps 6-9 until all the animals have been tested.
11. The recorded videos will be analyzed manually with the aid of a chronometer. Several parameters will be considered:

- Entries in closed arms and time spent in closed arms: May be used as inverse measures of anxiety that is to say, increased closed arm preference reflects high levels of anxiety (Carobrez and Bertoglio, 2005).
- Entries in open arms and time spent in open arms: May be used as inverse measures of anxiety, that is to say, reduced open arm avoidance reflects lower levels of anxiety (Carobrez and Bertoglio, 2005).
- Risk-assessment behavior: the frequency and the duration of head-dipping (downward movement of the head towards the floor while on the open arms) and stretch-attend posture (stretched posture with the head and two or three paws on the open arm and retraction to previous position) can be a direct measure of anxiety, i.e. increased risk-assessment behavior indicates higher levels of anxiety (Carola *et al.* 2004; Carobrez and Bertoglio, 2005).

3. Tail suspension test

The Tail Suspension Test is a rat behavioral test measuring “depression-like” behavior and learned helplessness. It will be used in the screening of depression-related behaviors.

It was conducted in a small 3-sided chamber in which the rats were suspended by the tail from a metal hook extending from the ceiling of the chamber, in such a position that they cannot escape or hold on to nearby surfaces. The test lasted for 6min and the immobility time measured during the final 4 minutes as nearly all rats will attempt to escape in the first 2 minutes. The total amount of immobility time (defined as the time during which the animal is hanging passively and motionless) were measured for each animal, and considered as an index of “depression-like” behavior (Frisbee, Brooks, Stanley and d'Audiffret, 2015).

All rats were carefully monitored for any adverse effects during the test and were removed quickly at any sign of unusual distress (i.e. constant vocalization or damage to their tails). The chambers were cleaned with 70% ethanol solution before and after each animal was tested.

1. The animals were suspended using a piece of adhesive tape (about 15 cm long) applied to the very end of the tail with 2-3 millimeters of tail remaining outside of the tape (Can *et al*. 2012), and attached to a metal wire hook.
2. Tape fragments used during the session were cut, marked, and prepared for the session.
3. The animals were brought into the testing room for one hour which allowed for acclimation of the animals before the test.
4. The tape fragments were adhered to the tails of the animals. The marked end of the tape was used, and made to stick to the tail and back on itself. The free end of the tape was attached on the suspension bar in a way that they do not obstruct the camera view.
5. Recording and identification of session was started before the rats were suspended.
6. The camera was close as possible in order to obtain the highest possible resolution of the animals.
7. At the end of the session (Which is typically six minutes), the animals were returned to their home cages and the tape carefully removed from each tail by gently pulling it off.
8. After returning the rats to their colony room, the fecal boli and urine were discarded from the collection trays and the apparatus wiped with a sterilizing solution.
9. The total time of mobility and immobility were analysed using ANOVA.

Animal Sacrifice and Brain extraction

The rats were euthanized according to ethical guidelines at the end of the study. Following the behavioural test, the animals were weighed, then anaesthetized under chloroform vapour (Ezejindu, Okafor and Anibeze, 2013).

The brain tissues of three of the animals in each group were prepared for biochemical analysis through the process of homogenization while the brain tissues of the remaining animals were fixed in 10% Neutral Buffered Formalin for 48hrs, (Sodium Phosphate Dibasic 6.5g, Distilled Water 900.0ml, Sodium Phosphate Monobasic 4.0g, Formaldehyde 37% 100.0ml) for further investigation using histology and immuhistochemical techniques.

Weighing of Animals and Relative brain weight

Body weight and brain weight of each animal were taken (Ijomone, 2011).

Relative brain weight (RBW) was calculated from body weight at sacrifice and whole brain weight as follows:

Relative brain weight = brain weight/body weight x 100

Biochemical analysis

The brain tissues were taken to Biochemistry Department for the analysis of oxidative stress markers (MDA) and antioxidant (SOD and GSH) through a process of homogenization. One gram of each animal tissue was added into 10ml of 0.9% normal saline each and homogenized at room standard temperature. After this, each of the samples were centrifuged at 3000rpm for 20min at room temperature. The supernatants were separated and stored for further analysis.

Tissue processing

Some fixed tissues were processed, sectioned and stained for H & E and crystal violet stain. After fixation, they were washed overnight under tap water so as to remove surplus fixatives, dehydrated to remove water and other substances. Following dehydration, tissues were cleared in xylene for two (2) hours after which infiltration was done in molten paraffin wax at a temperature of 60◦C for two (2) hours, each in two changes. When the paraffin wax cooled, sectioning was done (Feldman and Wolfe, 2014).

Hematoxyline and Eosin staining method

H and E staining technique will be used to detect histomorphological changes and necrotic bodies in eosinophilic appearance (Feldman and Wolfe, 2014). Coronal sections (30 mm) containing the brain tissue of interest will be mounted on slides and dried overnight, rehydrated, and stained for H and E as described by Drury and Wallington (1980) and Granado (2011). Microscopy will be conducted with an Olympus microscope (Tokyo, Japan) and images will be captured and processed by an attached eyepiece camera.

Nissl staining technique: some of the fixed tissues will be dehydrated in series of alcohol solutions and then embedded in paraffin wax blocks, cut into thin sections (4-8 micrometers) using a microtome and then mounted on glass slides. The paraffin will be removed from the tissue sections by soaking them in xylene or a xylene substitute, followed by a series of decreasing alcohol concentrations of (100%, 90%, 70%, and 50%). the tissue sections will be immersed in crystal violet staining solution for a few minutes to allow the dye to penetrate the tissue and bind to cellular components. excess staining solution will be rinsed from the

tissue sections with distilled water and differentiated in a differentiation solution- a mixture of alcohol and acetic acid. the sections will be rinsed with alcohol, followed by xylene or a xylene substitute, to dehydrate them. Finally, they will be mounted on a coverslip using a mounting medium, such as Canada balsam. Visualizing Nissl substance allows for the identification and characterization of neurons and their distribution.

Stereological quantification of neurons in the brain

The number of amygdala and hippocampus hematoxylin and eosin stained neurons will be counted unilaterally in every 4th sections of the tissue in every group (n¼4–6). The degeneration of neurons in the selected brain tissues will be assessed by counting H and E neurons of all experimental groups (n¼4–6) (de Olmos *et al*, 2009). The optical fractionator, Stereoinvestigator program (Microbrightfield Bioscience, Colchester, VT), will be used as described (Ares-Santos *et al.* 2012; Espadas *et al.* 2012) by an experienced observer unaware of treatment conditions. The outline of the brain tissues will be drawn at low power (x2) using defined anatomic landmarks (Ares-Santos *et al.* 2012; Baquet *et al.* 2009; Granado *et al.* 2008b), and the number of cells will be counted at higher power (x20 for the hippocampus and x100 for the amygdala and SNpc). At these magnifications, H and E stained cell bodies will be easily identified among the terminal degeneration in the amygdala, hippocampus and SNpc. To avoid double counting, neurons will be counted when their nuclei are optimally visualized, which occurred only in one focal plane. Some neurons with extremely faint signs of H and E will not be counted as H and E expressing neurons.

Immunohistochemistry

Some fixed tissues for immunohistochemical stains will be embedded in cryoprotectant. Serial coronal sections (50 μm) will be collected onto Superfrost microscope slides through the NAc (Bregma +1.96 mm to +0.61 mm) with a 150 μm inter-section distance. BCL-2 and TNF-α immunohistochemistry will be carried out on free-floating sections with standard avid in–biotin immune-cytochemical protocols (Granado *et al.* 2008a; Ortiz *et al.* 2010). Briefly, slides will be rinsed in phosphate-buffered saline (PBS) 3 times for 5 minutes each then blocked in 5% normal goat serum made in 0.3% triton-X solution. The slides will then be incubated overnight in primary antibody (Bcl-2 mouse monoclonal) at a 1:1000 concentration. The following day, slides will be rinsed in PBS 3 × 5 minutes then incubated for 2 hours in secondary antibody (goat anti-mouse) at a 1:250 concentration. Slides will be

rinsed in PBS 5 × 5 minutes. Once dry, slides will be cover-slipped with Vecta Shield Mounting Media with DAPI.

Images will be subsequently taken under light microscopy.

Quantitative assessment of immunoreactive expressions

Quantification of Bcl-2 and TNF-α expression and crystal violet staining in the brain will be carried out using pictures of the tissue sections taken with x400 lens in an optic microscope equipped with a Leica DFC 290 HD video camera. An image analysis system (Analytical Imaging Station) will be used to convert color intensities into a gray scale and to quantify the area of staining in the brain tissue as a proportion of pixels in the tissue that will show staining (stained area) in relation to total pixels in the brain tissue of interest (scanned area). We refer to this as proportional stained area (Darmopil, Martín, De Diego, Ares and Moratalla, 2009).

Photomicrograph and Image quantification

The processed tissues will be viewed under a Digital Light microscope and digital photomicrographs will be taken by an attached camera at x400, using OMAX software (Aluko and Umukoro, 2020).

NIH sponsored Image J software will be used for digital analysis of photomicrographs using the cell counter plugin (Erukainure *et al.* 2019).

Statistical Analysis and Data presentation

Data will be analyzed using SPSS version 27.0.1 software package. Mean and standard deviation will be obtained, differences between experimental groups will be obtained by independent sample t-test and One Way analysis of variance (ANOVA) will be used to compare values between groups followed by LSD (least square significant difference) post hoc test to compare the means of different group. Data will be expressed as Mean ± Standard error of mean and will be considered statistically significant when $P \leq 0.05$.

RESULTS

Table 4.1: Relative body weight of wistar rats.

	Groups	Mean± SEM	p-value	F-value
Initial Body Weight (KG)	Group A	190.50 ± 2.28		
	Group B	196.75 ± 2.42	0.741	1.454
	Group C	199.25 ± 3.21	0.365	

	Group D	200.62 ± 3.59	0.205	
	Group E	200.25 ± 2.55	0.243	
	Group F	200.00 ± 3.07	0.271	
	Group G	197.88 ± 3.19	0.570	
Final Body Weight (KG)	Group A	222.63 ± 3.10		
	Group B	199.80 ± 3.20	0.262	3.263
	Group C	227.13 ± 4.29	0.998	
	Group D	238.71 ± 4.69	0.556	
	Group E	217.20 ± 2.42	0.998	
	Group F	208.00 ± 16.09	0.703	
	Group G	220.61 ± 4.09	1.000	

Data were analyzed using One-way ANOVA followed by Turkey HSD multiple comparison, and data were considered significant at *, p<0.05, **, p<0.01, and ***, p<0.001

Table 4.2: The total weight of the brain in wistar rats.

	Groups	Mean ± SEM	p-value	F-value
Brain Weight (KG)	Group A	1.79 ± 0.06		
	Group B	1.42 ± 0.08	0.001***	10.233
	Group C	1.86 ± 0.12	0.963	
	Group D	1.91 ± 0.02	0.632	
	Group E	1.76 ± 0.04	0.998	
	Group F	1.77 ± 0.04	1.000	
	Group G	1.75 ± 0.04	0.995	

Data were analyzed using One-way ANOVA followed by Turkey HSD multiple comparison, and data were considered significant at *, p<0.05, **, p<0.01, and ***, p<0.001

Table 4.3: Neurobehavioral test.

	Groups	Mean ± SEM	p-value	F-value
Morris Water Maze Test (Sec)	Group A	15.50 ± 1.66		
	Group B	44.75 ± 3.47	0.000***	37.889
	Group C	14.25 ± 1.03	0.999	
	Group D	10.50 ± 0.65	0.508	
	Group E	28.75 ± 1.31	0.001***	
	Group F	23.50 ± 2.17	0.079	
	Group G	22.25 ± 2.14	0.192	
T-Maze Closed Arm	Group A	40.75 ± 4.87		
	Group B	87.75 ± 4.21	0.000***	22.800
	Group C	37.25 ± 2.78	0.995	
	Group D	31.25 ± 3.17	0.617	
	Group E	58.00 ± 4.65	0.067	
	Group F	46.25± 3.71	0.951	
	Group G	53.50 ± 3.66	0.292	
Tail Suspension Test (Immobility Time)	Group A	11.25 ± 1.49		
	Group B	33.75 ± 2.49	0.000***	41.122

	Group C	10.50 ± 0.65	1.000	
	Group D	9.25 ± 0.48	0.927	
	Group E	15.75 ± 1.25	0.234	
	Group F	13.75 ± 0.75	0.820	
	Group G	13.50 ± 0.87	0.880	

Data were analyzed using One-way ANOVA followed by Turkey HSD multiple comparison, and data were considered significant at *, p<0.05, **, p<0.01, and ***, p<0.001

Table 4.4: Antioxidant /Oxidative Stress markers.

	Groups	**Mean ± SEM**	**p-value**	**F-value**
SOD (U/ml)	Group A	17.34 ± 1.34		
	Group B	6.47 ± 0.03	0.000***	71.271
	Group C	18.79 ± 0.08	0.573	
	Group D	18.02 ± 0.04	0.495	
	Group E	12.12 ± 0.62	0.003**	
	Group F	10.77 ± 0.22	0.001***	
	Group G	16.96 ± 0.45	0.999	
GSH(U/I)	Group A	22.63 ± 2.53		
	Group B	6.59 ± 1.15	0.000***	42.808
	Group C	27.49 ± 0.49	0.148	
	Group D	26.85 ± 0.39	0.240	
	Group E	15.59 ± 0.83	0.038*	
	Group F	15.59 ± 0.28	0.030*	
	Group G	16.97 ± 0.05	0.081	
MDA(nmol/ml)	Group A	0.60 ± 0.01		
	Group B	3.07 ± 0.08	0.000***	389.801
	Group C	0.54 ± 0.01	0.959	
	Group D	0.55 ± 0.03	0.989	
	Group E	0.80 ± 0.05	0.150	
	Group F	0.80 ± 0.08	0.150	
	Group G	0.65 ± 0.30	0.972	

Data were analyzed using One-way ANOVA followed by Turkey HSD multiple comparison, and data were considered significant at *, p<0.05, **, p<0.01, and ***, p<0.001

Table 4.5: Cell Count on H and E.

	Groups	**Mean ± SEM**	**p-value**	**F-value**
Amygdala	Group A	41.00 ± 5.91		
	Group B	17.80 ± 2.87	0.998	4.016
	Group C	41.00 ± 7.77	0.144	
	Group D	43.00 ± 7.07	0.082	
	Group E	21.60 ± 1.63	0.144	
	Group F	29.60 ± 4.13	0.9232	
	Group G	39.00 ± 3.39	0.238	

Data were analyzed using One-way ANOVA followed by Turkey HSD multiple comparison, and data were considered significant at *, p<0.05, **, p<0.01, and ***, p<0.001

Amygdala- basolateral amygdala

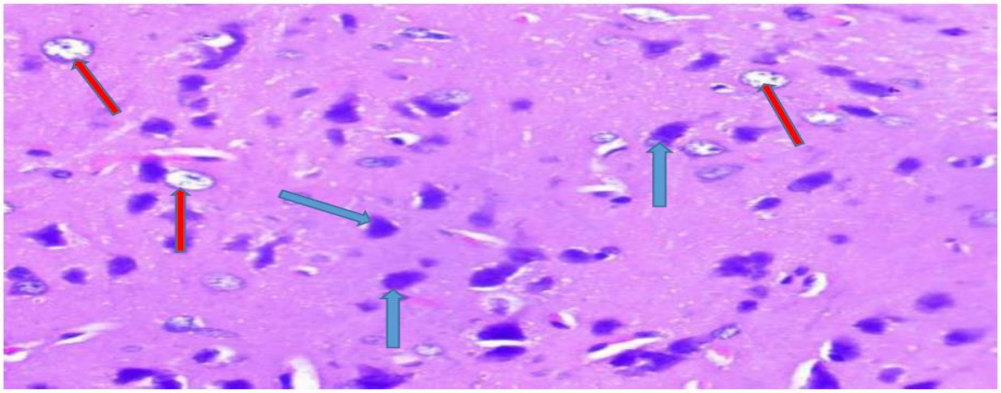

Figure 4: 1: Group A photomicrograph section of amygdala showing large multipolar neuronal cells (blue arrow), with mild vacuolated cells (red arrow). Stained by H and E (X400).

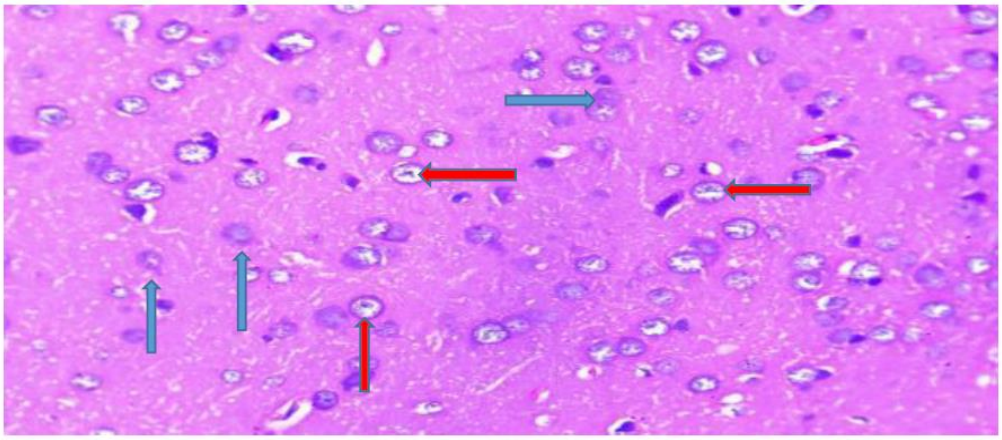

Figure 4.2: Group B photomicrograph of amygdala showing increased vacuolated cells (red arrow) and cellular shrinkage (blue arrow). Stained by H and E (X400).

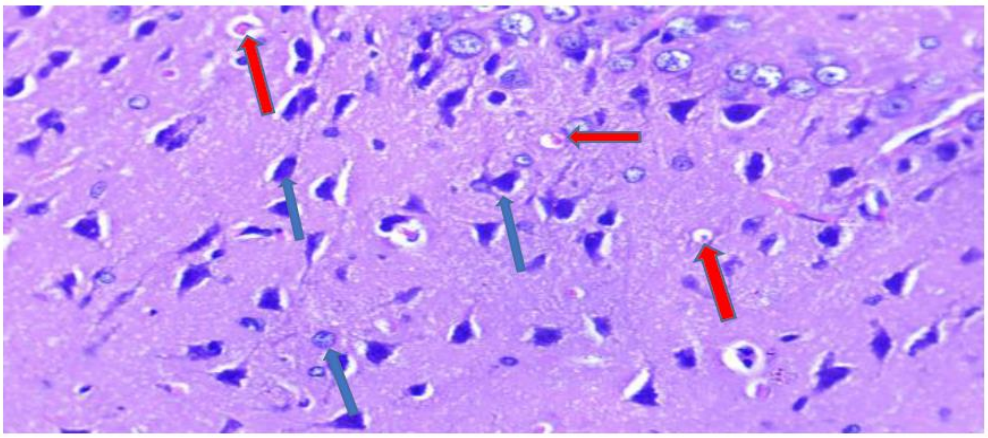

Figure 4.3: Group C photomicrograph of amygdala showing mild vacuolated cells (red arrow) and densely stained multipolar pyramidal cells (blue arrow). Stained by H and E (X400).

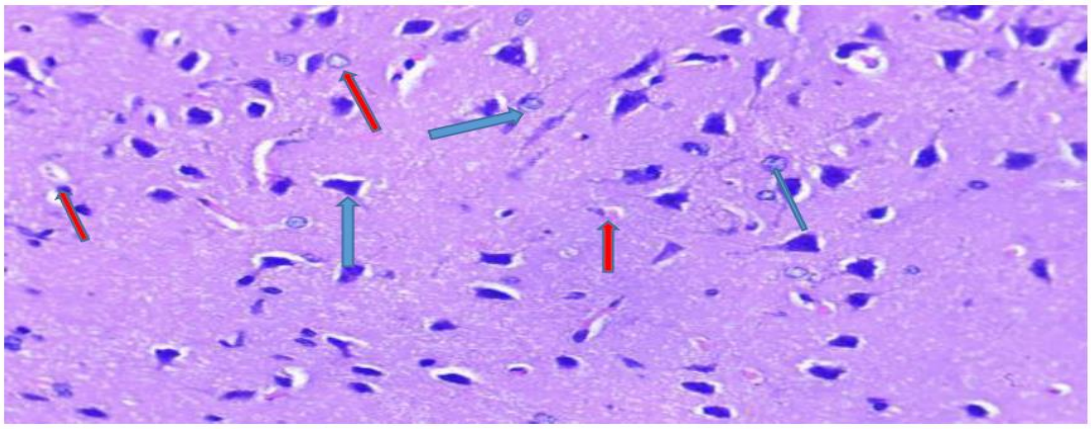

Figure 4.4: Group D photomicrograph of amygdala showing few vacuolated cells (red arrow) and densely stained multipolar pyramidal cells (blue arrow). Stained by H and E (X400).

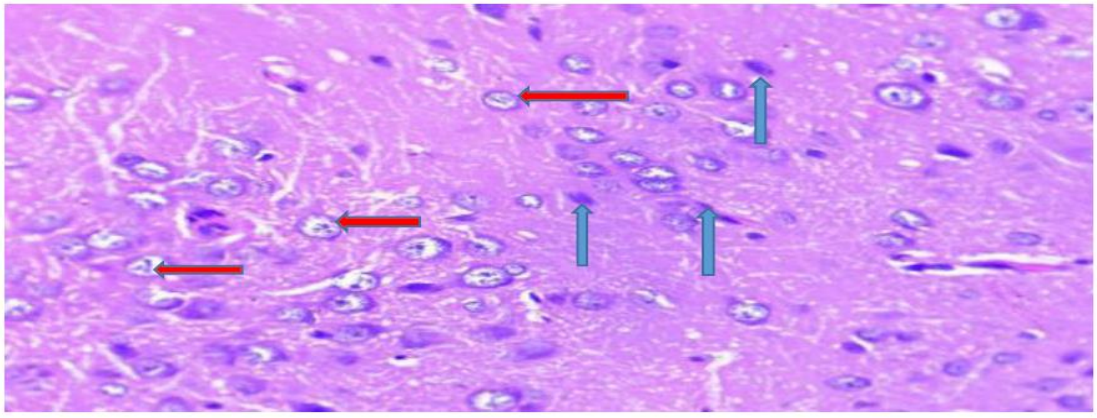

Figure 4.5: Group E photomicrograph of amygdala showing mild vacuolated cells (red arrow) and hyperchromatic cells (blue arrow). Stained by H and E (X400).

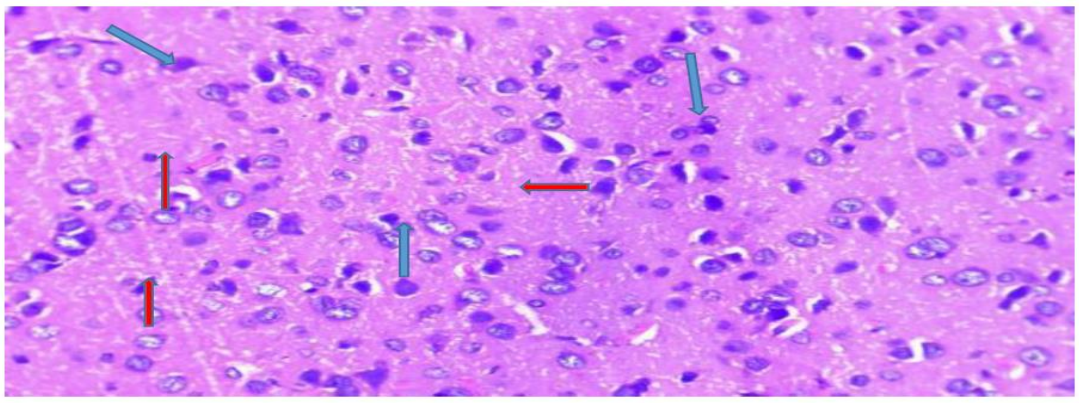

Figure 4.6: Group F photomicrograph of amygdala showing mild vacuolated cells (red arrow) and multipolar pyramidal cells (blue arrow). Stained by H and E (X400).

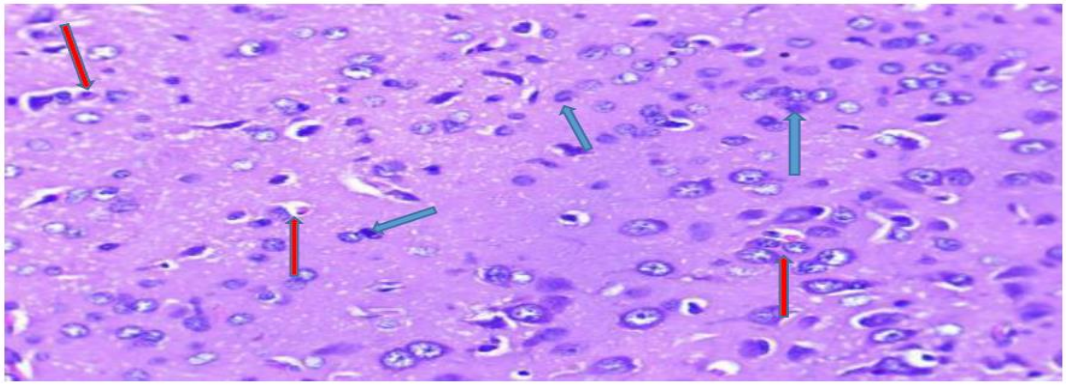

Figure 4.7: Group G photomicrograph of amygdala showing mild vacuolated cells (red arrow) and rare multipolar pyramidal cells (blue arrow). Stained by H and E (X400).

Table 4.6: Cell counts on CSV.

	Groups	Mean ± SEM	p-value	F-value
Amygdala	Group A	53.40 ± 11.76		
	Group B	30.40± 7.97	0.448	1.370
	Group C	53.80± 9.93	1.000	
	Group D	55.40 ±5.87	1.000	
	Group E	41.20 ± 7.13	0.950	
	Group F	44.80 ± 3.63	0.993	
	Group G	52.20 ± 5.05	1.000	

Data were analyzed using One-way ANOVA followed by Turkey HSD multiple comparison, and data were considered significant at *, $p<0.05$, **, $p<0.01$, and ***, $p<0.001$

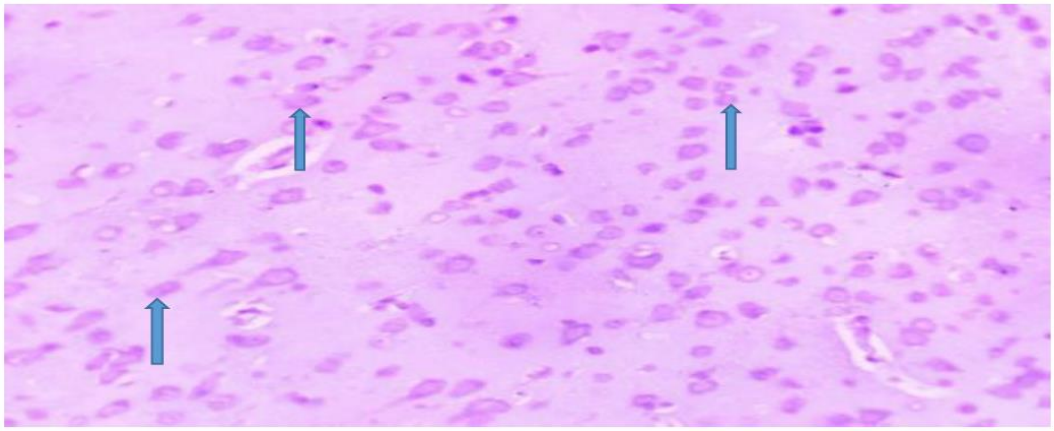

Figure 4.15: Group A photomicrograph of amygdala showing normal CSV positive basolateral amygdala cells (blue arrow). Stained by CSV (X400).

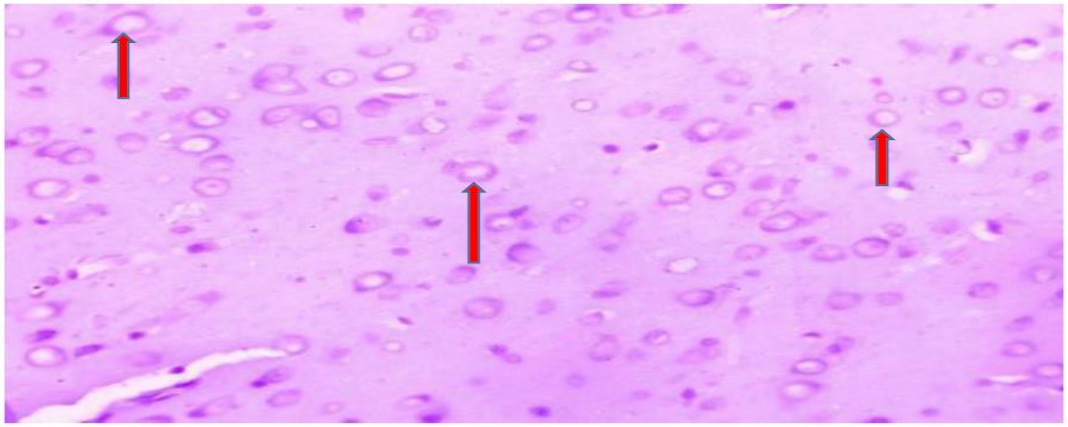

Figure 4.16: Group B photomicrograph of amygdala showing neuronal achromatic basolateral amygdala cells (red arrow). Stained by CSV (X400).

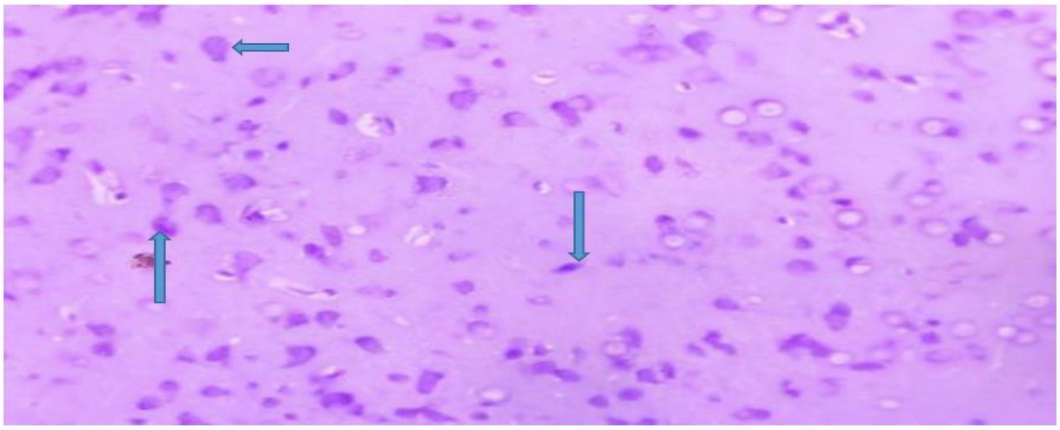

Figure 4.17: Group C photomicrograph of amygdala showing CSV positive basolateral amygdala cells (blue arrow). Stained by CSV (X400).

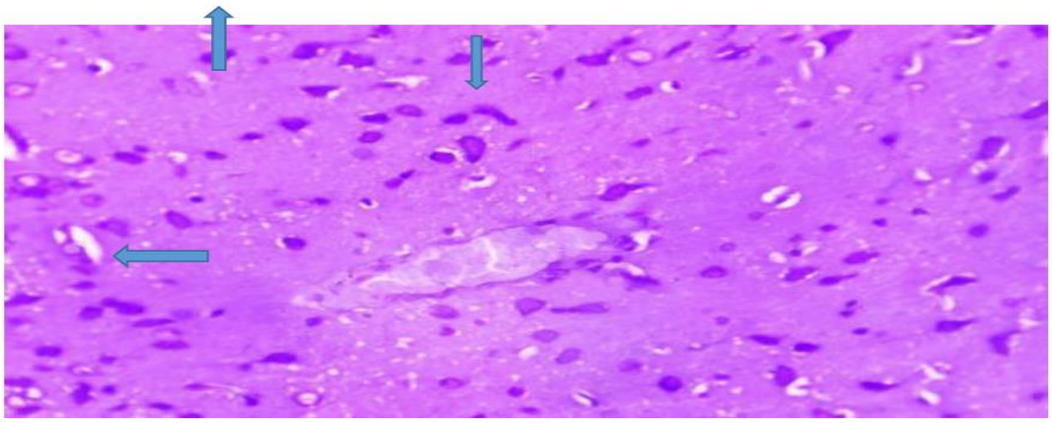

Figure 4.18: Group D photomicrograph of amygdala showing normal CSV positive basolateral amygdala cells (blue arrow). Stained by CSV (X400).

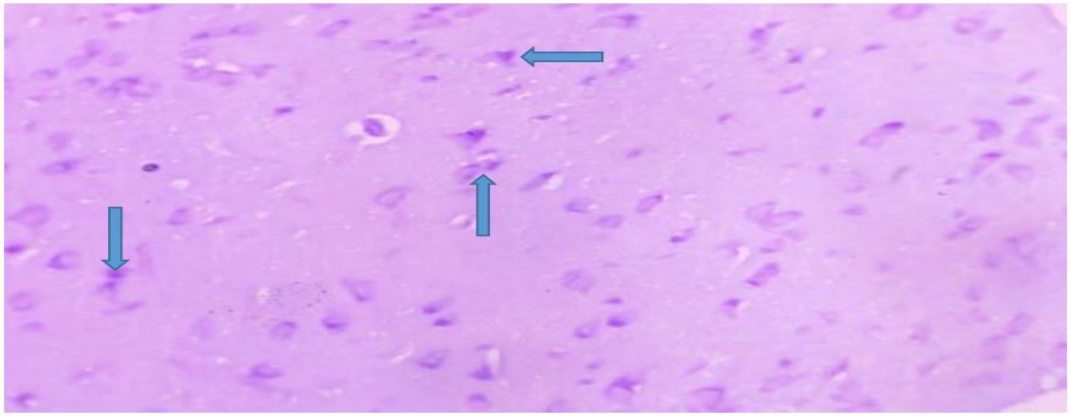

Figure 4.19: Group E photomicrograph of amygdala showing mild chromatolytic CSV positive basolateral amygdala cells (blue arrow). Stained by CSV (X400).

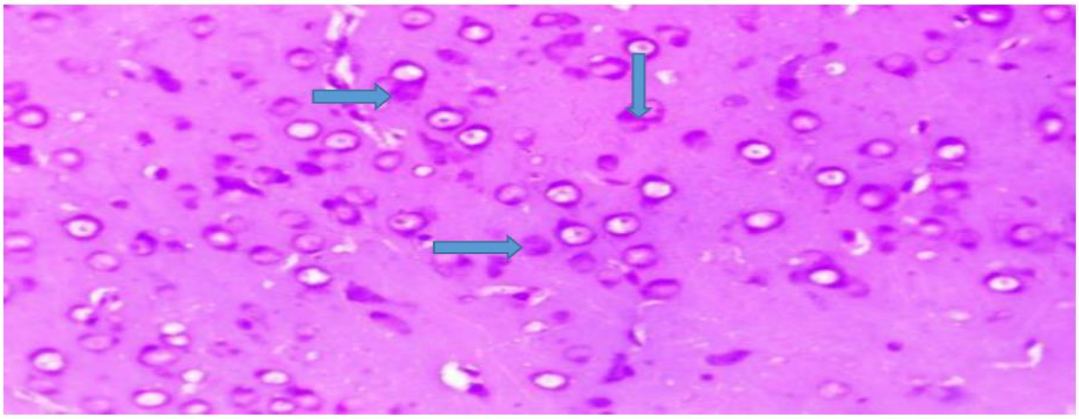

Figure 4.20: Group F photomicrograph of amygdala showing normal CSV positive basolateral amygdala cells (blue arrow). Stained by CSV (X400).

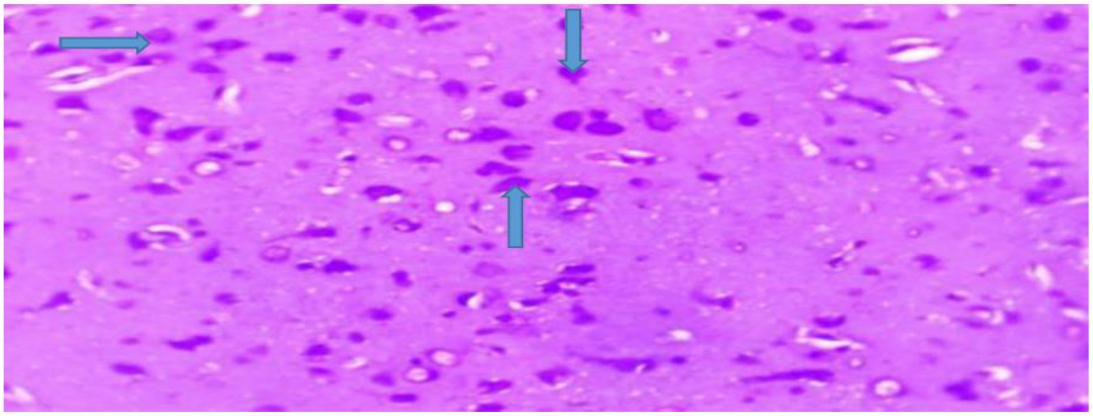

Figure 4.21: Group G photomicrograph of amygdala showing mild chromatolytic CSV positive basolateral amygdala cells (blue arrow). Stained by CSV (X400).

Table 4.1: Expressions of TNF.

	Groups	Mean ± SEM	p-value	F-value
Amygdala	Group A	43.20 ± 6.61		
	Group B	52.00 ± 4.30	0.301	1.585
	Group C	35.40 ± 5.46	1.000	
	Group D	33.40 ± 4.74	0.893	
	Group E	51.20± 5.35	0.349	
	Group F	48.40± 6.48	0.548	
	Group G	45.40 ± 7.30	0.768	

Data were analyzed using One-way ANOVA followed by Turkey HSD multiple comparison, and data were considered significant at *, $p<0.05$, **, $p<0.01$, and ***, $p<0.001$

Amygdala-TNF-α

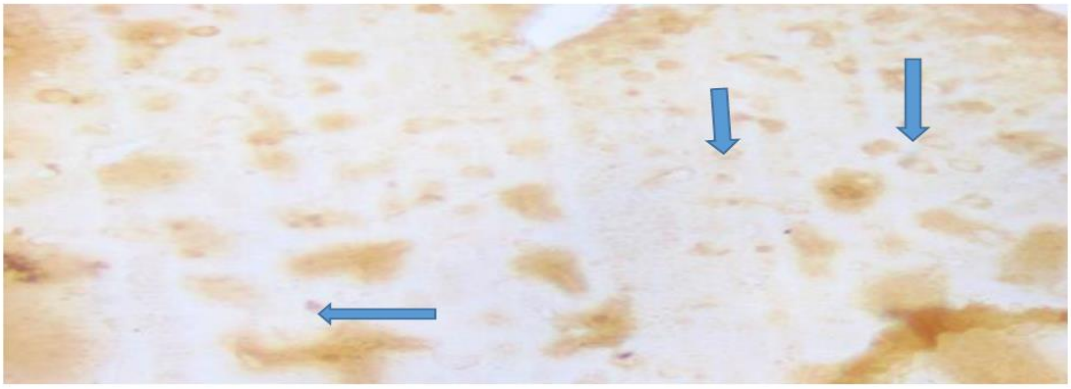

Figure 4.29: Group A photomicrograph of amygdala showing normal TNF-α expression in basolateral amygdala (blue arrow). Stained by TNF-α (X400).

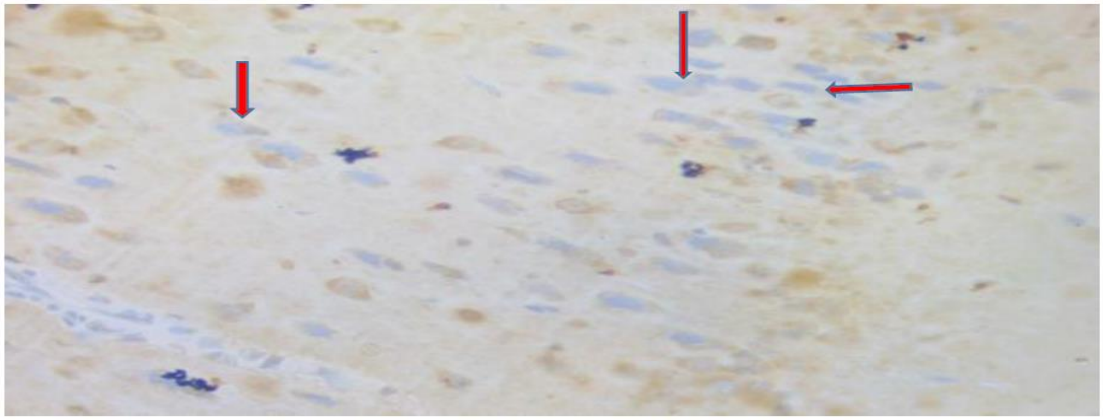

Figure 4: 30: Group B photomicrograph of amygdala showing increased TNF-α expression in basolateral amygdala (blue arrow). Stained by TNF-α (X400).

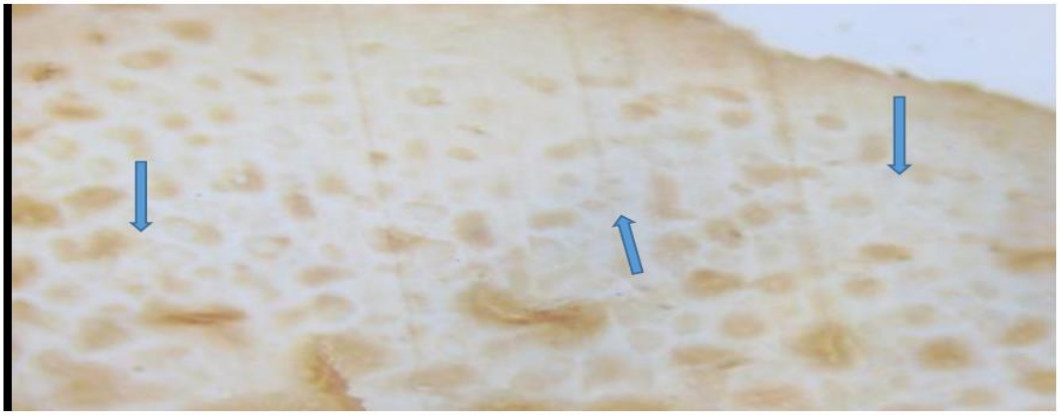

Figure 4.31: Group C photomicrograph of amygdala showing normal expression of TNF-α in basolateral amygdala (blue arrow). Stained by TNF -α (X400).

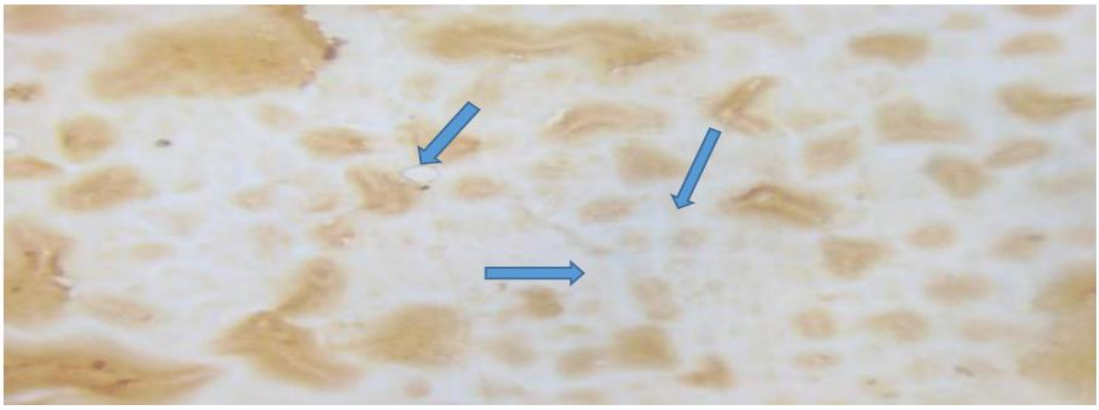

Figure 4.32: Group D photomicrograph of amygdala showing normal expression of TNF-α in basolateral amygdala (blue arrow). Stained by TNF-α (X400).

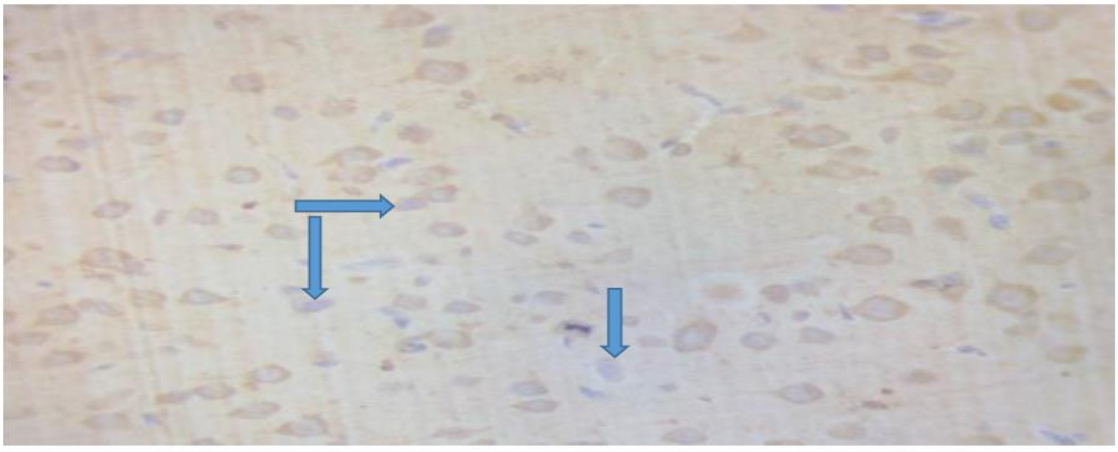

Figure 4.33: Group E photomicrograph of amygdala showing mild increase in the expression of TNF-α in basolateral amygdala (blue arrow). Stained by TNF-α (X400).

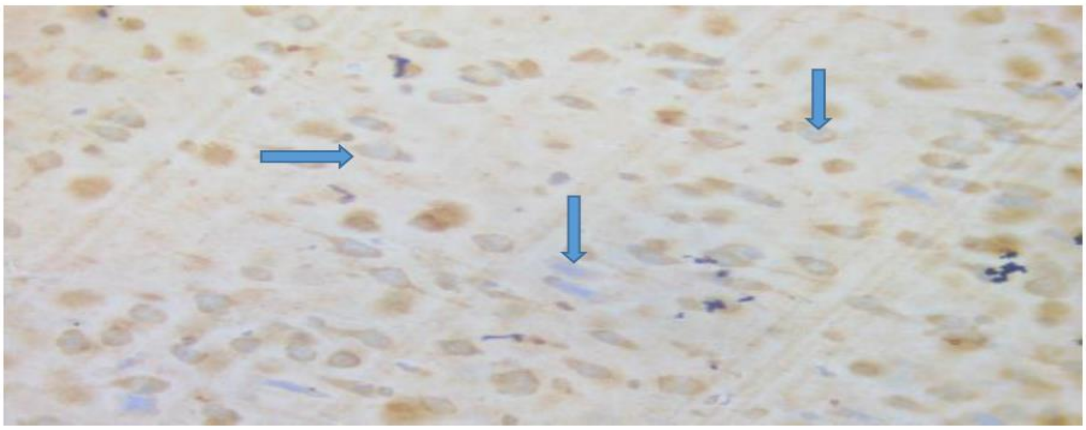

Figure 4.34: Group F photomicrograph of amygdala showing mild increase in the expression of TNF-α in basolateral amygdala (blue arrow). Stained by TNF-α (X400).

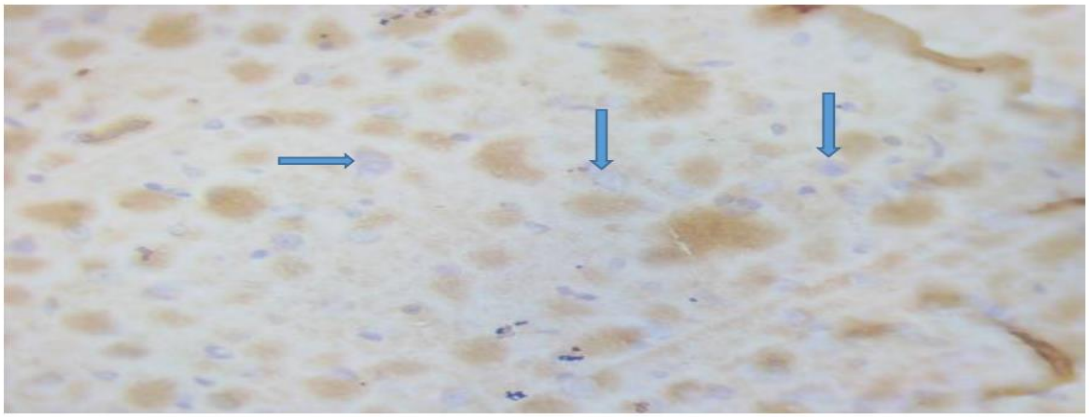

Figure 4.35: Group G photomicrograph of amygdala showing normal expression of TNF-α in basolateral amygdala (blue arrow). Stained by TNF-α (X400).

4.8: Cell Counts on BCL-2

	Groups	Mean ± SEM	p-value	F-value
Amygdala	Group A	67.60 ± 5.47		
	Group B	49.40 ± 7.28	0.000***	8.494
	Group C	87.80 ± 9.91	0.746	
	Group D	101.40 ± 6.75	0.014**	
	Group E	55.40 ± 4.03	0.000***	
	Group F	58.80 ± 4.83	0.001***	
	Group G	63.20 ± 4.87	0.004**	

Data were analyzed using One-way ANOVA followed by Turkey HSD multiple comparison, and data were considered significant at *, $p<0.05$, **, $p<0.01$, and ***, $p<0.001$

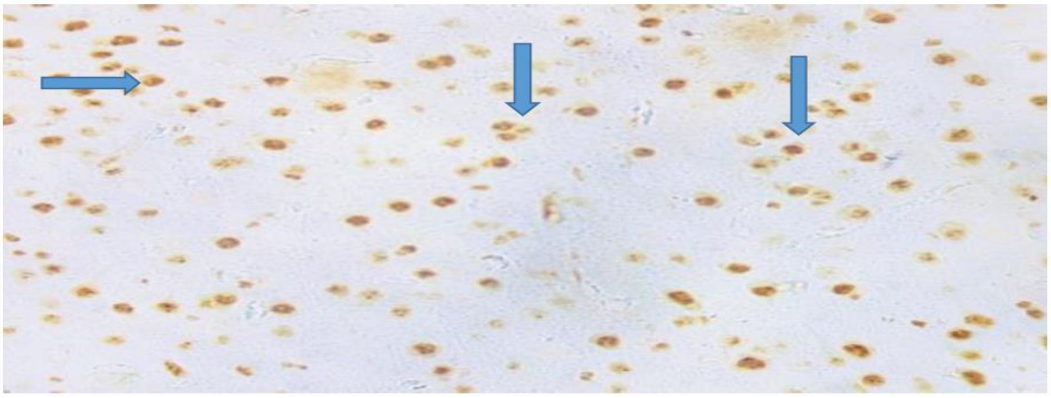

Figure 4.43: Group A photomicrograph of amygdala showing normal expression of BCL-2 in the basolateral region of amygdala (blue arrow). Stained by BCL-2 (X400).

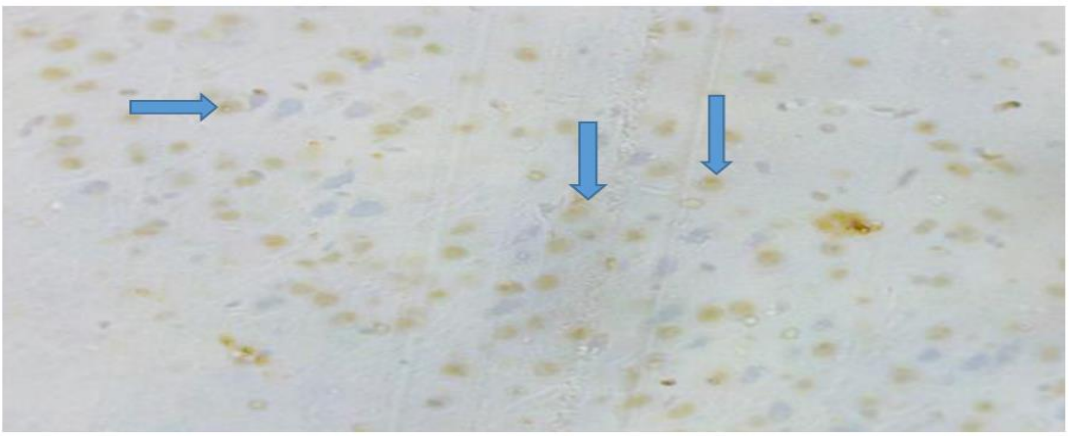

Figure 4.44: Group B photomicrograph of amygdala showing decreased expression of BCL-2 in the basolateral region of amygdala (blue arrow). Stained by BCL-2 (X400)

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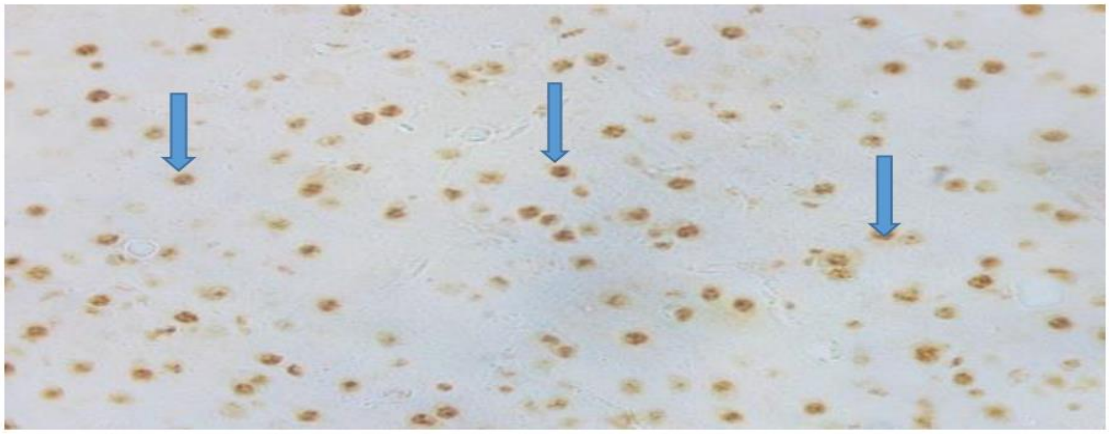

Figure 4.45: Group C photomicrograph of amygdala showing increase expression of BCL-2 in the basolateral region of amygdala (blue arrow). Stained by BCL-2 (X400).

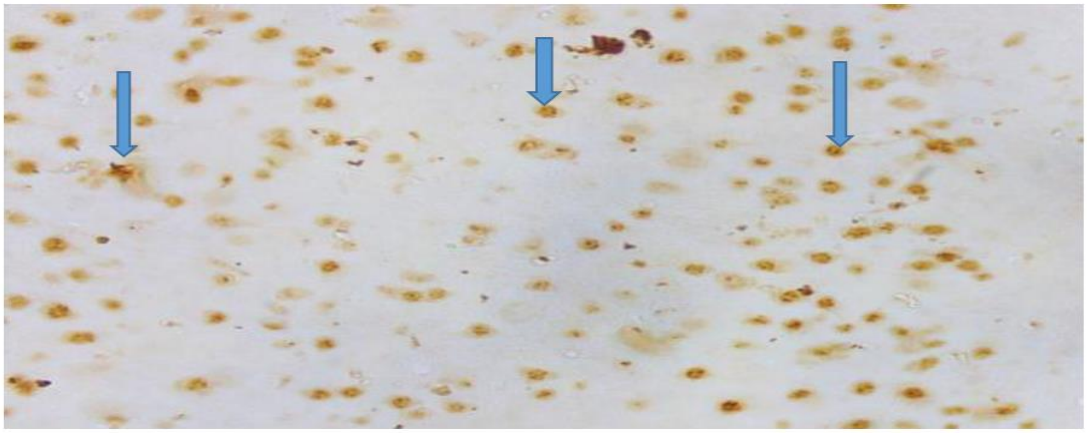

Figure 4.46: Group D photomicrograph of amygdala showing increase in the expression of BCL-2 in the basolateral region of amygdala (blue arrow). Stained by BCL-2 (X400).

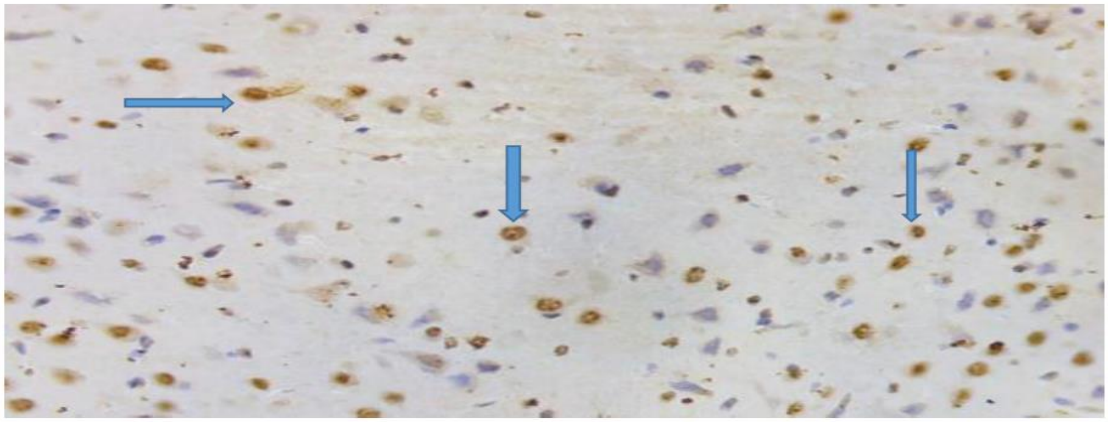

Figure 4.47: Group E photomicrograph of amygdala showing significant increased expression of BCL-2 in the basolateral region of amygdala (blue arrow). Stained by BCL-2 (X400).

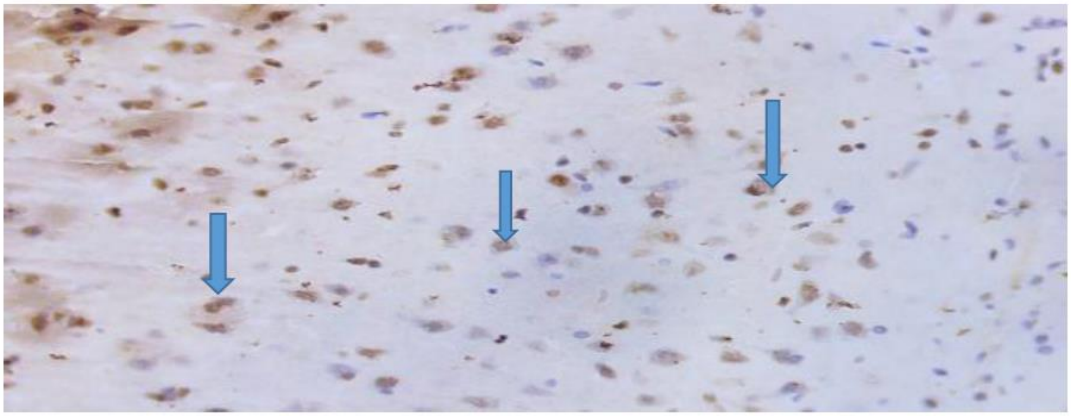

Figure 4.48: Group F photomicrograph of amygdala showing increased expression of BCL-2 in the basolateral region of amygdala (blue arrow). Stained by BCL-2 (X400).

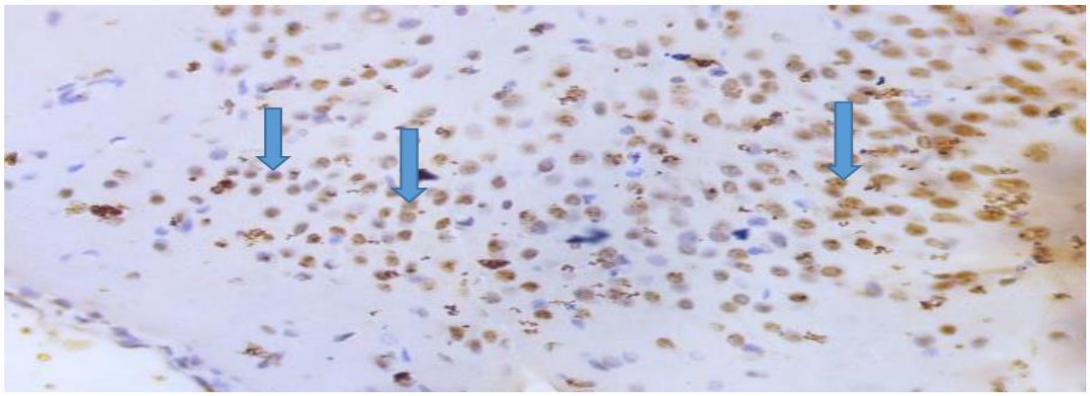

Figure 4.49: Group G photomicrograph of amygdala showing increased expression of BCL-2 in the basolateral region of amygdala (blue arrow). Stained by BCL-2 (X400).

DISCUSSION

Meth remains a significant public health concern with known neurotoxic and neurocognitive effects to the user (Courtney and ray, 2014). It triggers a disruption of the monoaminergic system, abuse of meth leads to negative emotional states including depressive symptoms during drug withdrawal (Courtney and Ray, 2014).

Meth has been shown to significantly increase oxidative stress markers such as lipid peroxidation, reactive oxygen species formation and glutathione oxidation in the brain (Al-Hakeim et al. 2022; Shokrzadeh et al. 2015) which is agrees with the findings of this work. Its exposure has been found to cause significant depression, anxiety and cognition impairment in experimental animals which was also noted in this study. This study demonstrated that meth increased MDA, TNF-α and a reduced SOD, GPx activities, this finding is in consistence with the work of Keshavarzi *et al*. (2019), this has been shown to be from induction of oxidative stress, which is responsible for severe cognitive deficits in animal studies (Brinks *et al.* 2023). Impairment in hippocampus-dependent cognitive abilities occurs in various conditions associated with elevated levels of oxidative stress. (Ambrogini *et al*. 2016; Armenta-Resendiz, 2022).

A large body of evidence gathered in animal models has documented that antioxidant compounds, including vit. E, can buffer or prevent the decline in hippocampal LTP (a long-lasting increase in synaptic transmission efficacy proposed as a cellular substrate for mammalian learning and memory (Baghcheghi *et al.* 2019).

Vit. E, known as the first line of defense against the peroxidation of lipids (Jomova *et al*.2023), has a well-documented antioxidant and anti-inflammatory features (Azzi, 2018).

Baghcheghi *et al.* (2019) demonstrated that Vit. E prevented the LTP impairment and neuronal apoptosis in the hippocampus of juvenile hypothyroid rats proving its neuronal protection efficacy.

This study demonstrated that vit. E is neuroprotective as evidenced by improvement in neuro-behavioural status of animal group F, with associated changes in antioxidant status in group F which is consistence with the works of Crouzin *et al* 2010; Khanna et al. 2005; Numakawa *et al*. 2006; Pace *et al.* 2010; Roghani and Behzadi, 2001.

Vit C is considered to be a vital antioxidant molecule in the brain and it's crucial role in neuronal maturation and differentiation, myelin formation, synthesis of catecholamine, modulation of neurotransmission and antioxidant protection is well supported by the evidences presented Kocot *et al.* (2017). It protects the brain from oxidative damage in various models of neurodegeneration (Harrison and May, 2009).

Study by Shah, Yoon, Kim and Kim (2015) reported the therapeutic efficacy of vit C in response to glutamate-induced excitation, resulting in energy depletion and apoptosis in the hippocampus of the developing rat brain, co-treatment of vit C with glutamate decreased brain glutamate levels and reversed the changes induced by glutamate in the hippocampus. This study agrees with the findings of this study on animal group E. Kangisser *et al*. (2021); Olajide *et al*. (2017); Sil *et al*. (2016) also demonstrated that vit C is neuroprotective.

Zare *et al.* (2019), demonstrated that co-supplementation of vit E and vit C possess synergistic effects and significantly decreased MDA levels in sodium metabisulphite-induced damage, this is in agreement with the report of this study. Study by Kalender *et al*. (2010), also proved the synergistic effect of vit C and E.

The amygdala consists of a group of heterogeneous nuclei located in the medial portion of the temporal lobe and is involved in multimodal information processing important for emotional recognition and behavior (Benarroch, 2015). Its complex structure includes basolateral, centromedial, and cortical nuclear complexes that have extensive connections with several cortical and subcortical structures (Benarroch, 2015). It is involved in a wide range of normal behavioral functions and psychiatric conditions (LeDoux, 2007). Several neurologic disorders and psychiatric disorders affects amygdala, including Alzheimer disease, temporal lobe

epilepsy, anxiety, and depression (Benarroch, 2015), as seen in this study which demonstrated significant increase in anxiety and depression like behaviour in group B.

In this study, both the initial and final body weight showed statistical non-significant difference in groups B, C, D, E, F and G when compared to group A. This does not agree with the work of Nwodo *et al.* (2023) who demonstrated that oral administration of meth caused significant reduction in weight of Wistar rats when compared to parenteral administration. Also, Bortolato *et al*. (2010) in agreement with their previous findings (Bortolato *et al*. 2009), showed that meth injected rats displayed significant reductions in body weight in comparison to saline-treated controls. Study by Smith *et al.* (2006), also reported less maternal weight gain during pregnancy in meth exposed group with associated low birth weight when compared to control group.

This study showed that the total brain weight has statistical significant decrease in group B while groups C, D, E, F and G showed non-significant difference when compared to A. This finding disagrees with the work of Ijomone (2011) and Thanos *et al.* (2016), who found no statistically significant difference in mean relative brain weight across the treatment groups compared to control.

The Morris Water Maze test presents statistical significant increase in groups B and E, while groups C, D. F and G showed non-significant difference when compared to group A. This agrees with work of Ijomone (2011) who also demonstrated statistically significant impaired spatial memory following treatment with meth when compared to the other groups on Morris water maze and as seen in this study, administration of compound with antioxidant activity improved learning and memory deficit of experimental animals. Works by Garmabi, Mohaddes, Rezvani, Mohseni and Khastar. (2022), Golsorkhdan *et al.* (2020) and Hadizadeh-Bazaz, Vaezi and Hojati. (2021) also demonstrated significant impairment in learning and spatial memory following meth intoxication which got improved following administration of different compounds with antioxidant and anti-inflammatory effects.

The Elevated plus Maze Test (closed arm) showed statistically significant increase in group B, while groups C-G showed non-significant difference when compared to group A. This is used to measure anxiety level in the experimental animals. Study by Hughes, Lowther and van Nobelen. (2011), demonstrated that vitamins C and E separately and combined together decreased anxiety-related behavior, suggesting that decrease in anxiety produced by the

vitamins may have arisen from their antioxidant properties. They demonstrated no evidence of synergism between vitamins C and E in their effects. Henderson (2015) and Soliman, Khattab and Habil. (2018), also noted improvement of anxiety related behaviour following treatment with vit C, which agrees with this current study.

The tail suspension Test for depression like attitude showed statistical significant increase in immobility time in group B while groups C-G showed non-significant when compared to group A. This is supported by Silva *et al*. (2014), who showed that meth significantly increased the immobility time in the tail suspension test at 3 and 49 days post-administration. The findings of Goyal, Gupta and Verma (2016) and Lobato *et al.* (2010), supported this study by demonstrating that vit C and E has significant effect in decreasing immobility time on tail suspension test in experimental animals.

The SOD shows statistical decrease in groups B, E and F while groups C, D and G were statistically non-significant when compared to the experimental group A. The GSH-Px showed statistical decrease in groups B, E and F while in groups C, D and G were statistically non-significant when compared to group A. The MDA showed statistical increase in group B, while groups C-G were non-significantly different when compared to group A.

The above findings are consistent with the findings of Hadizadeh-Bazaz *et al*. (2021) who also demonstrated that administration of meth caused significant decrease in in levels of SOD, GSH-Px, and an increase in MDA levels which were reversed by curcumin (compound with antioxidant effect) when compared to meth group as seen in this study. The work of Jayanthi, Ladenheim and Cadet (1998) shared similar findings with this study except an opposite effect on SOD. They investigated the effects of meth-induced toxicity on cortical and striatal antioxidant defense systems on mice brain and found out that meth significantly increased SOD, induced decrease in GSH-Px and MDA. This was also demonstrated by Al-Hakeim *et al.* (2022).

Kim *et al.* (2016) investigated the protective effects of vit. E on meth-induced neurotoxicity in mice and found that pre-treatment with vit. E significantly reduced meth-induced oxidative stress markers and protected against neuronal damage in the brain. This shows that vit. E could be a potential therapeutic option to prevent meth-induced brain damage. The effect of vit. E on Amygdala and synergistic action of vit. C and E against meth has not been evaluated as seen in this work. Chen, Wang, Chen, Li and Zhou (2008) made similar findings following

investigation of the protective effects of folic acid on meth-induced neurotoxicity in rats, suggesting that folic acid supplementation could be a potential therapy for meth-induced neurotoxicity. Work by Azimzadeh *et al.* (2023) agrees to the result of this study.

Several studies with different compounds with antioxidant properties demonstrated their effects against oxidative stress in the brain as seen in this work. These includes the work of Kargar and Noshiri (2023) that used Alpha-lipoic acid against meth induced neuronal damage, Moshiri *et al.* (2018), using vit. B12 against brain damages caused by meth in mice and found out that administration Vit. B12 30 minutes before meth elevated the levels of GSH in striatum and cerebral cortex of the brain and returned it to normal levels compared to the meth group, Wawrzyniak *et al.* (2013), using vit. E, vit. C, and β-carotene on oxidative stress status in rats subjected to exercise induced oxidative stress and recorded a reduction in MDA level when compared to control group, Garmabi *et al.* (2022), using erythropoietin against meth induced neurooxicity and consequent reduction in MDA level, Shokrzadeh *et al*. (2015) using propofol against meth-induced oxidative stress in Wistar rats, Srigiridhar and Nair (2000), α-tocopherol, ascorbic acid or a combination of both against oxidative damage on the gastrointestinal tract of iron-deficient rats during Fe repletion, though noted that ascorbic acid alone does not protect the gastrointestinal tract against Fe-induced oxidative stress.

Riffel *et al.* (2018) provided evidence that administration of vit. C and E, given alone or in combination, prevented changes in pro-oxidant and antioxidant markers in the spinal cord of chronic constriction injury (CCI) rats, thus agreeing with synergistic actions of these vitamins. Poli, Aparna, Madduru and Motireddy (2022) also demonstrated these by using Vit. C and E against liver and kidney toxicity of Cadmium in rats.

LaTorre *et al.* (2021) showed that although there is much in vivo and in vitro evidence of vit. E antioxidant and protective abilities, there are still conflicting results for its use as a treatment for neurodegenerative diseases that speculate that vit. E, under certain conditions or genetic predispositions, can be pro-oxidant and harmful.

This study demonstrated that both H and E and CSV cell counts on Amygdala region showed no statistical significant difference in groups B-G when compare to group A. this is at variance with the work of Ijomone (2011), which showed significant reduction in Nissl substance staining which were reversed by Kolaviron. Naseer *et al*. (2009), found significant

neurodegeneration following CSV cell count on ethanol treated group which was reversed by co treatment with vit. C. Study by Zhang *et al.* (2022) agrees with the findings of Naseer et al. (2009) following investigation of the effects of vit. E on ferroptosis of hippocampal neurons in epileptic rats.

Naseer *et al*. (2011) and Rafati, Noorafshan, Jahangir, Hosseini and Karbalay-Doust (2018), working on anti-inflammatory and antiapoptotic effects of antioxidants (vit. C and E) respectively found there is significant increase in CSV expressing cells following antioxidants treatment. These disagrees with the findings of this study.

TNF-α expressions on Amygdala region in this study shows statistical non-significant difference in groups B-G when compare to group A. This varies with the study done by Permpoonputtana and Govitrapong. (2013) investigated whether meth caused inflammatory effects in human dopaminergic neuroblastoma SH-SY5Y cells, and found that meth significantly increased the levels of TNF-α and that melatonin significantly decreases TNF-α levels caused by meth. Hadizadeh-Bazaz *et al.* (2021) also agreed on significant increase in TNF-α following meth induced neurotoxicity in rats which antioxidants rich compound (curcumin) decreased significantly in the CAI area of hippocampus. Gonçalves *et al*. (2010), investigated the hippocampal dysfunction induced by an acute high dose of meth and preventive effect of indomethacin focusing on the inflammatory process and changes in several neuronal structural proteins. They observed that meth caused a significant increase in TNF-α which indomethacin- an anti-inflammatory agent reduced. This is at variance with this study which despite having an increase in TNF-α in group B is not statistically significant. Azimzadeh *et al.* (2023), also demonstrated significant increase in TNF-α.

BCL2 expression on Amygdala region shows statistically significant decrease in group B, increase in groups D, E, F and G while group C shows non-significant difference when compare to group A. Study by Ma *et al*. (2014), found that meth exposure-induced autophagy early on and progressively changed into apoptosis in a time-dependent manner which was suggested to be mediated by upregulating Beclin1 expression and disrupting the balance between Beclin1 and Bcl-2. They demonstrated significant decrease in BCL-2 following meth exposure which agrees with the findings of this study. This was supported by works of Cadet, Ordonez and Ordonez. (1997); Imam *et al.* (2001); Wang, Yen, Yin, Chi and Lee, (2008). Decrease in Bcl-2 have been associated with various pathological processes.

Naseer *et al*. (2011) and Rafati *et al.* (2018), working on anti-inflammatory and antiapoptotic effects of antioxidants (vit. C and E) respectively found that they significantly decreased TNF-α and increased BCL-2, which is in partial agreement with this index study that found non- significant decrease in TNT-α but significant increase in BCL-2.

Several studies found that meth induced decrease in BCL-2 can be reversed by antioxidants and pharmacological agents, as seen in works of (He, Xu, Yang, Zhang and Li, 2004; Huang *et al.* 2017; Long *et al.* 2017; Moshiri *et al*. 2018; Nopparat, Porter, Ebadi and Govitrapong, 2010; Zeng *et al*. 2021) which are in agreement to the outcome of this study. In agreement to this, the work of Zhao *et al.* (2016), who studied the effects of dl-3-n-butylphthalide (NBP) on Meth-mediated neurotoxicity in SH-SY5Y neuroblastoma cells found that meth induced significant cell injury and apoptosis by up regulation of intracellular ROS, decrease of Bcl-2/Bax ratio and increase of c-caspase-3/caspase-3 ratio. The meth-associated alterations were attenuated and inhibited by NBP, suggesting that NBP might be used as a therapeutic agent in against Meth-induced neuronal injury in the central nervous system.

This decrease in BCL-2 level following meth exposure has also been recorded in other organs of the body and the protective effect of antioxidants documented. Salimi *et al.* 2023, demonstrated that meth exposure decreased Bcl-2 expression in cardiac cells, suggesting an increase in cardiac apoptosis, and that Vit.E administration increased Bcl-2 expression, suggesting a protective role in cardiac muscle cells.

Both vit. C and E have been well documented to possess positive anti apoptotic effects. The work of Amanpour, Khodarahmi and Salehipour (2020), Investigated the mechanism of Cadmium (Cd)-induced apoptosis and the protective effects of vit. E on rat testes. They found out that Vit. E significantly increased Bcl-2 in the rats' testes receiving Cd. Haendeler, Zeiher and Dimmeler. (1996). Found that Lipopolysaccharide induced apoptosis and necrosis of human umbilicial venous endothelial cells in a time-dependent manner. The increase in apoptosis correlated with a reduction in Bcl-2. Combination of vit. C and E completely inhibited lipopolysaccharide-induced apoptosis, whereas vit. C or E alone was less effective. The reduction of lipopolysaccharide-induced apoptosis by vit. C and E was paralleled by an increase in Bcl-2. This gives support to the synergistic effect of vit. C and E as an anti-apoptotic agents as also seen in this study.

In conclusion**,** this study concludes that treatment with meth results in weight loss, though not statistically significant and vit. C and E treatment improves weight gain. Also, treatment with meth resulted in a decline in total brain weight and vit. C and E improved the total brain weight. Treatment with meth also resulted in increased MDA activities in the amygdala and a decline in SOD and GSH levels which were ameliorated by treatment with vit. C and E. Further, treatment with meth resulted in decreased cell count on H and E stain, CSV stain and TNF-α stain, though not statistically significant. Treatment with vit. C and E offered some protection. Treatment with meth showed significant decrease in BCL-2 level which were improved by vit. C and E.

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