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Neuroprotective and Therapeutic Effects of Vitamin B Complex on the Cerebral Cortex of Methaphetamine Induced Adult Male Wistar Rats

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Abstract

Methamphetamine (commonly referred to as meth, crystal, ice, or crank) is a powerful, highly addictive synthetic central nervous system (CNS) stimulant. It belongs to the amphetamine class of drugs but is more potent, faster-acting, and more neurotoxic than amphetamine. This study investigated the protective role and therapeutic effects of vitamin B complex against methamphetamine-induced neurotoxicity in adult male Wistar rats. A total forty- two rats (weighing 130g-175g) were used for the study. Fourteen rats were used for acute toxicity test while twenty-eight rats were randomly assigned into seven groups (A–G, n = 4). Group A served as the control, Groups B and C received methamphetamine at 2 mg/kg and 10 mg/kg respectively, while Groups D and E were administered vitamin B complex at 50 mg/kg and 100 mg/kg respectively. Groups F and G received combined treatments, with Group F administered 2 mg/kg of methamphetamine plus 50 mg/kg of vitamin B complex, and Group G receiving 10 mg/kg of methamphetamine plus 100 mg/kg of vitamin B complex (high dose). Sub-chronic oral administration was carried out, and parameters assessed included body weight and neurobehavioural test for memory and cognitive activity. Oxidative stress markers (GSH, SOD, and MDA) evaluated redox balance while Nissl substance were analyzed to assess cerebral cortex neural integrity. Rats treated with methamphetamine alone (Groups B and C) exhibited significant weight loss ($p < 0.01$), increased relative organ weights, and marked histopathological alterations characterized by reduced normal pyramidal cell counts and increased inflammatory cell infiltration, indicating severe neuronal degeneration. Conversely, rats treated with vitamin B complex, either alone or in combination (Groups D–G), demonstrated significant weight gain comparable to the control, and preservation of cerebral histoarchitecture. These groups showed increased numbers of normal pyramidal cells with reduced inflammatory changes compared to methamphetamine-only groups, suggesting enhanced neuroprotection. In conclusion, vitamin B complex mitigated methamphetamine-induced neuronal degeneration, improved body weight, and preserved cortical integrity. These findings highlight the potential therapeutic role of vitamin B complex in reducing stimulant-induced brain injury and support its possible application as a neuroprotective agent.

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Background of the Study

Methamphetamine, a powerful central nervous system stimulant and N-methylated derivative of amphetamine [1]. While its pharmaceutical form has limited use in treating attention-deficit/hyperactivity disorder, the drug has high abuse potential, severe health risks, and unpredictable behavioral effects Methamphetamine (METH) can be ingested orally, injected, snorted, or smoked (Anglin M. D., Burke C., Perrochet B., Stamper E. and Dawud-Noursi S. 2000). Methamphetamine's street names include crank, crystal meth, ice, speed (Anglin et al.2000) and mkpurummiri ,nnukwuahia, kpotomkpo in the eastern part of Nigeria, amongst others.

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).

Rising use of methamphetamine continues to impose devastating effects on individuals, families, and healthcare systems worldwide (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).

Chronic METH exposure produces oxidative stress, excitotoxicity, neuroinflammation, and neuronal apoptosis (Yang et al, 2018 and Fiorentini et al., 2021). Neuroimaging reveals reduced dopamine levels, decreased dopamine transporter (DAT) density, and microglial activation in the striatum, resembling Parkinson's disease pathology (Jon Stoessl A. 2011). These changes underpin deficits in cognition, memory, and psychomotor function. (Yang et al., 2018). Clinically, METH abuse is linked with paranoia, hallucinations, tremor, delusions, and psychosis, while withdrawal features depression, anxiety, hypersomnolence, and agitation. Some symptoms resolve quickly, but cognitive and affective deficits often persist, correlating with ongoing neuronal damage (Siefried et al, 2020 and Fiorentini et al., 2021). Clinical manifestation of meth addiction depends on the dose and duration of use (Paulus and Stewart, 2020).

Treatments for meth use disorder are currently limited to behavioural 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, Koustova, 2023).

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). Increasing body of evidence has shown that METH-induced neurotoxicity is dependent upon the production of reactive species, irrespective of METH mode of entry into the neuron (Davidson et al., 2001; Escubedo et al., 2005). Excessive dopamine release drives oxidative stress, lipid peroxidation, mitochondrial dysfunction, and excitotoxicity, while hyperthermia worsens injury (Ares-Santos et al 2013 and Fiorentini et al, 2021). These processes impair executive function and motor coordination, with meta-analyses confirming deficits in working memory, processing speed, and impulse control (Yang et al., 2018).

Another critical dimension of METH abuse is nutritional compromise. Substance use disorders often cause deficiencies in essential vitamins, especially B vitamins, which are vital for energy metabolism and neurotransmitter synthesis (Cruickshank and Dyer 2009). METH disrupts utilization of thiamine (B1), pyridoxine (B6), and cobalamin (B12), exacerbating oxidative stress and neurotoxicity. Addressing these deficits through supplementation may provide therapeutic benefit. Vitamin B complex, consisting of eight water-soluble vitamins, plays central roles in neuronal repair, neurotransmitter regulation, and homocysteine metabolism (Morris, 2003 and Eltony,2016). Deficiencies are strongly associated with depression, anxiety, and cognitive decline (Kennedy, 2016). Experimental studies indicate that B vitamins attenuate apoptosis, gliosis, and oxidative damage in models of METH exposure and diabetes-related neurodegeneration (Eltony,2016 and Moshiri,2018).

This study aims to investigate the neuroprotective and therapeutic effects of vitamin B complex administration on cerebral cortex of methamphetamine-induced adult male Wistar rats.

Materials And Methods

Study Area and Duration of the Study

This scientific investigation was carried out in the Animal House of the Department of Human Anatomy, College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus, Anambra State, Nigeria. The experimental animals were allowed to acclimatize for a period of 14 days, after which the test substances were administered for 28days; the entire experiment lasted for six weeks.

Ethical Approval

Approval for this study was obtained from the Nnamdi Azikiwe University- Animal Research Ethics Committee with the approval number, NAU/AREC/2025/0086. This was carried
out in strict compliance with "National Institutes of Health Guide for the Care and Use of Laboratory Animals"

Materials for the Study

In the course of this investigation the following materials were utilized;

- Adults male wistar rats: The experimental animals
- Iron cages and finisher mash)
- Water: For drinking and washing.
- Troughs: Used for feeding with iron nettings: use to house the experimental animals. Saw dust (litter),
- Animal feed (grower g of the rats
- Laboratory coat and gloves: For protection
- Electronic weighing balance: Used to carry out various measurements
- Syringes and cannula: Used in the administration of the extracts.
- Measuring cylinder: For fluid measurement.
- One stopwatch: used for recording time
- Clean caps of 15 ml conical tubes, 55cm wide 2- mm thick metallic wire,
- Water bath: To incubate the brain tissues in water at a constant temperature

Experimental Design and Protocols

EXPERIMENTAL DESIGN

S/N	TREATMENT GROUP	DOSAGE	REFRENCES	DURATION
Group A	Control	Food and Water ad libitum	Okafor et al., 2025	4 weeks
Group B	Low Dose Meth	2 mg/kg	Okafor et al., 2025	4 weeks
Group C	High dose meth	10 mg/kg	Okafor et al., 2025	4 weeks
Group D	Low dose Vitamin B complex	50 mg/kg	Okafor et al., 2025	4 weeks
Group E	High dose Vit. B Complex	100 mg/kg	Okafor et al., 2025	4 weeks
Group F	Low dose meth + Low dose Vit. B complex	2 mg/kg + 50 mg/kg	Okafor et al., 2025	4 weeks
Group G	High dose meth + High dose Vit. B complex	10 mg/kg + 100 mg/kg	Okafor et al., 2025	4 weeks

Forty-two (42) adults male Wistar rats weighing 130-175 g were procured from Research Enterprise Farms, University of Ibadan Oyo State. The animals were kept in the Animal House in the Department of Anatomy, Nnamdi Azikiwe University, Nnewi campus. They were allowed to acclimatize over a period of two weeks. The animals were housed in well-ventilated iron cages under normal temperature (27-31 ℃) throughout the period of experiment. They were fed with starters and growers mash from Guinea Feeds PLC, Nigeria and tap water in clean cages. All the animals were treated in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health Guide for the care and use of laboratory Animals (NRC 2011)

Acquisition of Experimental Substances

Procurement of Methamphetamine

Crystal Methamphetamine was procured from the national drug law enforcement agency {NDLEA}

Procurement of Vitamin B Complex

Vitamin B-complex was purchased from a licensed pharmaceutical supplier in Nnewi, Anambra state Nnewi, Anambra State. It was administered to the experimental animals orally using cannula.

Reconstitution of Test Substances

Methamphetamine and vitamin B complex were freshly prepared before administration. METH was reconstituted in distilled water following Madden et al.'s method, and vitamin B-complex prepared according to the manufacturer's instructions; stock solutions were freshly prepared daily, and dosages calculated relative to body weight (mg/kg).

A stock solution of methamphetamine was obtained by dissolving 4 g of the compound in 400 mL of distilled water, yielding a concentration of 10 mg/mL. Similarly, vitamin B complex was reconstituted by dissolving 1000 mg in 100 mL of distilled water, giving a concentration of 10 mg/mL. The required doses for each experimental group were calculated according to the body weight of the animals and administered orally using a calibrated syringe fitted with an oral cannula to ensure accurate delivery.

Acute Toxicity Test (Ld50) of Methamphetamine and Vitamin B Complex

The acute toxicity test of methamphetamine and vitamin B was carried out in the Faculty of Pharmaceutical Sciences. Nnamdi Azikiwe University Agulu Campus, Anambra State. In this study, a total of 14 Wistar rats were used. METH was reconstituted in distilled water following Madden et al.'s method , and vitamin B-complex prepared according to the manufacturer's instructions; stock solutions were freshly prepared daily, and dosages calculated relative to body weight (mg/kg). Acute oral toxicity (LD_{50}) of METH and vitamin B-complex was assessed using Lorke's method , modified by Doera et al., and involved two phases. In phase one, three groups of two rats each received 10, 100, and 1000 mg/kg, and were observed for 72 hours for toxicity signs and mortality. In phase two, four groups of two rats each received 1200, 1600, 2900, and 5000 mg/kg. The LD_{50} of METH was determined as 32.5 mg/kg, while vitamin B-complex showed no mortality up to 5000 mg/kg, indicating a wide margin of safety.

Animal Grouping and Administration

A total of twenty-eight rats were randomly divided into seven groups and these groups were labeled A-G. Group A served as the control and received normal feed (grower mesh) and distilled water for twenty-eight (28) days while group B - G served as the test groups. The administration was performed orally with graded dose prepared according and weighed to determine the quantity to be administered. Methamphetamine and vitamin B-complex were administered twice in a day between the hours of 7:00am and 4:00pm for a period of twenty-eight (28) days.

Group B was administered 2 mg/kg of methamphetamine, while Group C received 10 mg/kg methamphetamine. Group D was treated with 50mg/kg vitamin B complex, and Group E received 100mg/kg vitamin B complex

Group F was administered a combination of 2mg/kg methamphetamine and 50mg/kg vitamin B complex, whereas Group G received a combination of 10mg/kg methamphetamine and 100mg/kg vitamin B complex

Mode of Administration

An oral gavage needle was utilized as the method of oral administration whereby the needle is gently passed through the mouth down into the stomach and the substances are delivered directly into the stomach. The animal was gently held by the skin of the back of its neck, thereby exposing the mouth. This method ensures accurate dosing and minimal stress to the animals during the process.

The liquid form of the drugs was generated by dissolution of the substances in water. The animals in each group were administered in a well-ventilated room with the specific dose of drug required as obtained in the LD50. The administration of methamphetamine and vitamin B complex was maintained for a specified length of time depending on the group. This was necessary to ensure the accurate concentration of the drugs within the experimental time.

Behavioural Function Tests

Behavioural testing was performed between 09:00 and 15:00 h under quiet laboratory conditions.
Animals were allowed to acclimatize to the testing room for at least 30 minutes before each
session. All apparatus were cleaned with 70% ethanol and allowed to dry between animals to
remove olfactory cues

Morris Water Maze Test

The Morris water maze is widely used to study spatial memory and learning. Animals are placed in a pool of water that is colored opaque with powdered non-fat milk or non-toxic tempera paint, where they must swim to a hidden escape platform. Because they are in opaque water, the animals cannot see the platform, and cannot rely on scent to find the escape route. Instead, they must rely on external/extra-maze cues. As the animals become more familiar with the task, they are able to find the platform more quickly. Developed by Richard G. Morris in 1984, this paradigm has

become one of the "gold standards" of behavioral neuroscience (Nunez, 2008).

Biochemical Analysis

The oxidative stress parameters assayed from the brain tissue homogenates included malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD). These biomarkers were selected as they are reliable indicators of lipid peroxidation and antioxidant defense status in experimental neurotoxicity models (Janero, 1990; Ayala *et al*., 2014).

Tissue Homogenization

One gram of each brain tissue was weighed and homogenized in 10 mL of 0.9% normal saline at room temperature using a mechanical homogenizer. The homogenates were centrifuged at 3000 rpm for 20 minutes, and the supernatant was collected and stored at 2 °C until further biochemical assays (Ohkawa *et al.,* 1979).

Indication

MDA has been widely used for many years as a convenient biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids because of its facile reaction with thiobarbituric acid. MDA is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress and the antioxidant status in cancerous patients. When MDA level goes up in brain disease condition, the antioxidants are expected to go down. Antioxidant is an analyte frequently used to assess the antioxidant status of biological samples and can evaluate the antioxidant response against the free radicals produced in a given disease. In the normal in-vivo state, glucose and oxygen are the two substrates for energy metabolism in the brain but glucose is the only significant substrate.

Lipid peroxidation

Lipid peroxidation (LPO) was investigated with the method described by Ohkawa *et al.,* 1979. One milliliter (1mL) of 10% chilled (w/v) trichloroacetic acid (TCA) was added to one milliliter of 10% homogenate, incubated at 37°C for 10mins and centrifuged at 2,500 rpm for 15mins at room temperature. One milliliter of 0.67% thiobarbituric acid (TBA) was added to 1ml of supernatant and kept in a boiling water bath for 10-15mins. After cooling, 1ml of distilled water was added to it and absorbance was taken at 530nm. The results were expressed as nmol MDA/h/g tissue.

Non-enzymatic antioxidant: Reduced glutathione

Glutathione (GSH) will be investigated with the method described by Ellman (1959). One milliliter of 5% TCA (w/v) was added to 1ml of 10% homogenate. The suspension was left for 30mins and centrifuged at 2500rpm for 15mins. 0.5ml of supernatant was taken and 2.5ml of 5'5'-dithionitrobenzoic acid (DTNB) was added. The suspension was shaken thoroughly and read at 412nm. The results were expressed as µmol/g tissue.

Enzymatic antioxidant: Superoxide dismutase

Superoxide dismutase (SOD) was investigated with the method described by Kakkar *et al*., 1984. A total of 650µl of sodium pyrophosphate buffer was added to 50µl of brain supernatant fraction; 50µl phenazine methosulfate (PMS), 150µl of nitroblue tetrazolium (NBT), and 100µl nicotinamide adenine dinucleotide phosphate (NADPH) was added and the mixture vortexed thoroughly. The reaction mixture was incubated for 90s and 500µl glacial acetic acid was added to stop the reaction. Two milliliter of n-butanol was added, vortexed thoroughly. It was kept at room temperature for 10min. Absorbance was measured at 560nm. The results were expressed in terms of µmol/min/mg protein.

Tissue Processing

For easy study of sections under microscope, the tissues were passed through several processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining.

After weighing the organs, a small part of the hippocampus and prefrontal cortex were cut out and immediately fixed in 10% formal saline in order to preserve the various constituents of the cells in their normal micro anatomical position and to also prevent autolysis and putrefaction. Tissue sections were produced by normal histochemical methods of dehydration, clearing, impregnation, embedding, sectioning and staining (with H&E) after fixation. The micrographs of the relevant stained sections were subsequently taken with the aid of a light microscope.

Statistical Analysis.

Data was analyzed using SPSS version 27.0.1 software package. Mean and standard deviation were obtained and one-way analysis of variance (ANOVA0 was used to compare values between groups. Data was expressed as Mean ± Standard Deviation (SD) and then considered statistically significant when $P \leq 0.05$

Results and Discussion

Physical and Behavioural Changes

At the beginning of the experiment, all the animals were healthy and active. During the two weeks of acclimatization, they eat well and their stool was normal and they also adapted to their environment well and quickly. On exposure to the methamphetamine, the following behaviours were noticed;

- Reduced motor function
- Reduced body weight
- Reduced appetite for food
- Showed signs of aggressiveness

Body Weight Changes

As shown in Table 1, Group A experienced a weight gain of +25.43 g (from 150.00 ± 0.00 g to 175.43 ± 0.42 g), while Group B had a weight loss of −4.95 g and Group C showed a further weight reduction of −10.36 g. Conversely, Groups D, E, F, and G recorded weight gains of +15.43 g, +17.33 g, +17.41 g, and +19.92 g, respectively. Statistically, Groups B and C differed significantly from Group A (*a*), whereas Groups C, D, E, F, and G differed significantly from Group B (*b*).

Table 1: Showing the Weight Changes between Groups

Group	Initial Weight (g)	Final Weight (g)	Weight Change (g)
A	150.00 ± 0.00	175.43 ± 0.42	25.43*
B	153.07 ± 0.12	148.12 ± 0.05	−4.95a#
C	148.01 ± 0.08	137.65 ± 0.32	−10.36ab#
D	149.42 ± 8.50	164.85 ± 0.00	15.43ab*
E	151.33 ± 3.74	168.66 ± 0.03	17.33ab*
F	143.84 ± 2.65	161.25 ± 6.43	17.41ab*
G	149.38 ± 1.31	169.30 ± 0.54	19.92ab*

Values represent Mean ± SEM. a represents a significant difference when compared to Group A; b represents a significant difference when compared to Group B; * represents a significant increase when final weight is compared to initial weight; # represents a significant decrease when final weight is compared to initial weight.

Histological Cell Count in the Cerebral Cortex

In Table 2 using H&E stain, the data on normal cells (NC) in the cerebral cortex revealed a notably higher mean count in Group D (124.00 ± 1.73), while Group C showed the lowest (11.00 ± 1.00). Group A, serving as the reference, recorded 87.00 ± 1.16. Groups B and C showed significantly lower NC counts compared to Group A, while Groups D to G displayed various levels of statistically significant differences in comparison to Groups A and B. The inflammatory cells (IC) count was highest in Group C (78.00 ± 1.00) and lowest in Group F (15.00 ± 1.16). Compared to Group A (20.00 ± 1.53), Groups B and C exhibited significantly elevated inflammatory cell counts. Conversely, Groups E, F, and G showed significantly reduced IC counts when compared to Group B.

Table 2: Cerebral Cortex Cell Counts using H&E Stain

Groups	Normal cells (NC)	Inflammatory cells (IC)
A	87.00±1.16	20.00±1.53
B	29.00±1.00a	75.00±2.52a

C	11.00±1.00ab	78.00±1.00a
D	124.00±1.73ab	39.00±2.52ab
E	45.00±1.53ab	16.00±1.16b
F	65.00±1.73ab	15.00±1.16b
G	45.00±1.53ab	16.00±2.08b

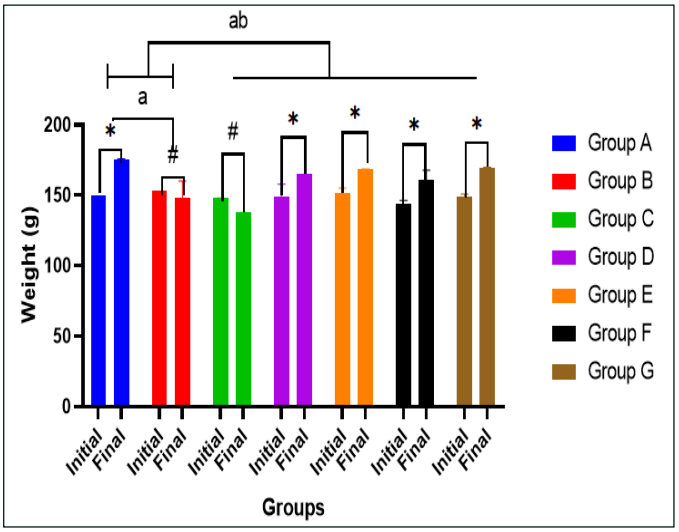
Values represent Mean ± SEM. a represents a significant difference when compared to Group A; b represents a significant difference when compared to Group B.

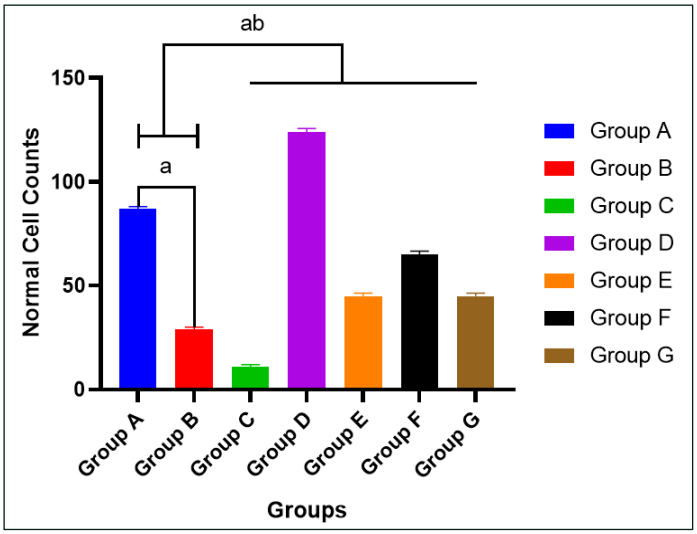
Using cresyl violet stain, the count of normal pyramidal neurons (NPN) was highest in Group G (48.00 ± 0.58) and lowest in Group C (23.00 ± 0.58). Groups B and C had significantly lower NPN counts than Group A (41.00 ± 0.58). At the same time, Groups D, E, and G demonstrated significantly higher counts compared to Group B, with Group G showing the highest neuronal count among all groups. The pyknotic pyramidal neuron (PPN) counts were highest in Group C (42.00 ± 0.58) and lowest in Group F (8.00 ± 0.58). Groups B and C recorded significantly elevated PPN counts when compared to Group A (10.00 ± 0.58), while Groups D, E, and F showed significantly reduced PPN values compared to Group B. Group G also showed a significantly different value from Group B but recorded the highest value among the treatment groups (Table 3).

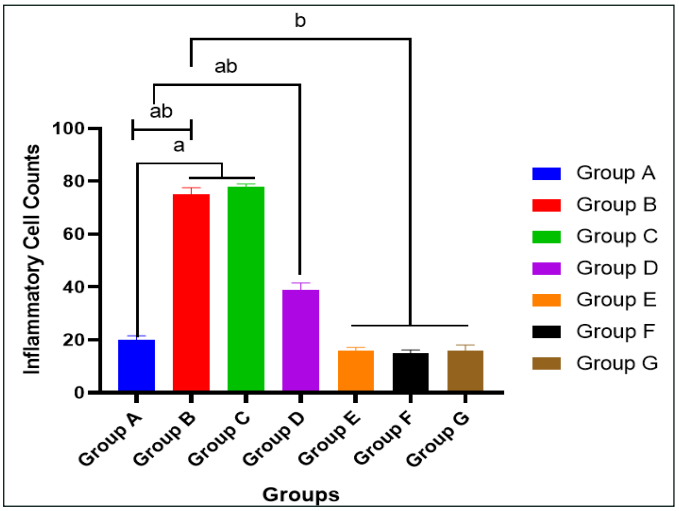
Table 3: Cerebral Cortex Neuronal Counts using Cresyl violet

Groups	Normal Pyramidal Neurons (NPN)	Pyknotic Neurons (PPN)
A	41.00±0.58	10.00±0.58
B	24.00±0.58a	41.00±0.58a
C	23.00±0.58a	42.00±0.58a
D	42.00±0.58b	12.00±1.16b
E	42.00±1.16b	13.00±0.58b
F	30.00±0.58ab	8.00±0.58b
G	48.00±0.58b	20.00±1.00ab

Values represent Mean ± SEM. a represents a significant difference when compared to Group A; b represents a significant difference when compared to Group B.







Cerebral Cortex Neuronal counts using H and E

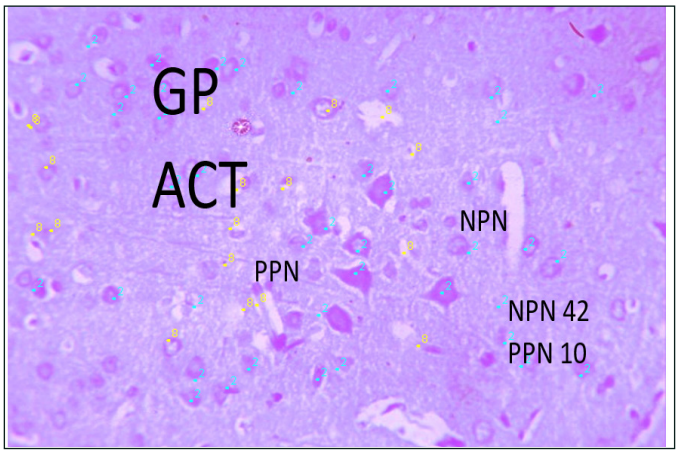


Plate 1: Photomicrograph of A CT control section of cerebral cortex (x100(x400)(H/E) shows nissil body with active prominent pyramidal neuron (PN) in CA1 region, numbering normal pyramial neuron (NPN) 42 and pyknotic pyramaidal neuron (PPN) 10

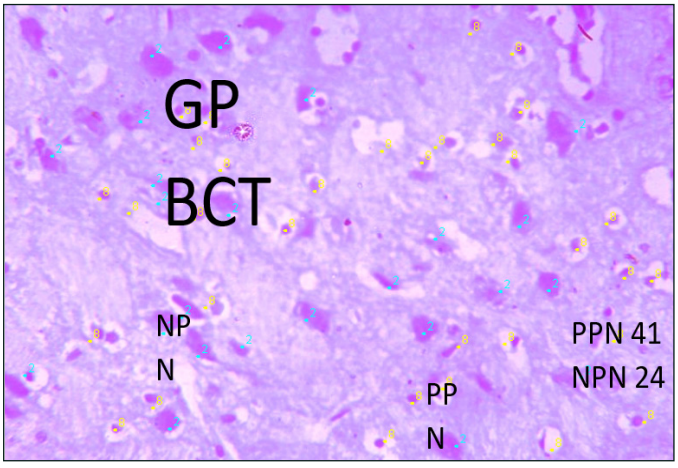


Plate 2: Photomicrograph of BCT section of cerebral cortex indused with low dose metha (x100(x400) (H/E) shows moderate degeneration with nissil body with moderate pyknotic pyramidal neuron (PPN) in CA1 region, numbering normal pyramial neuron (NPN) 24 and pyknotic pyramaidal neuron (PPN) 41

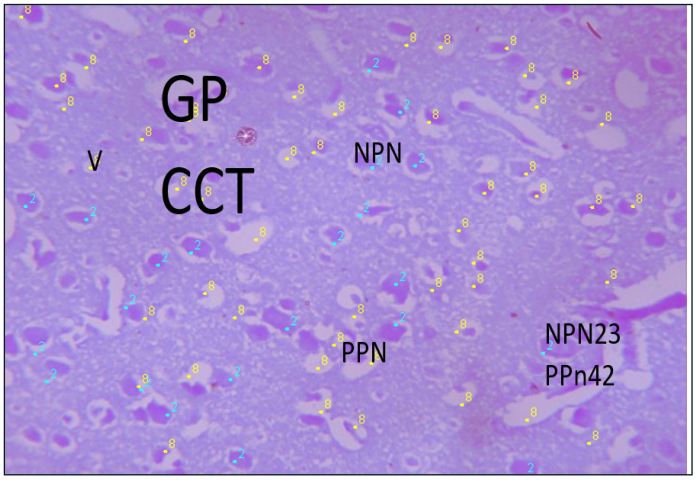


Plate 3: Photomicrograph of CCT section of cerebral cortex indused with HIGH dose metha (x100(x400) (H/E) shows moderate degeneration with with moderate pyknotic pyramidal neuron (PPN) in CA1 region, numbering normal pyramial neuron (NPN) 24 and pyknotic pyramaidal neuron (PPN) 41

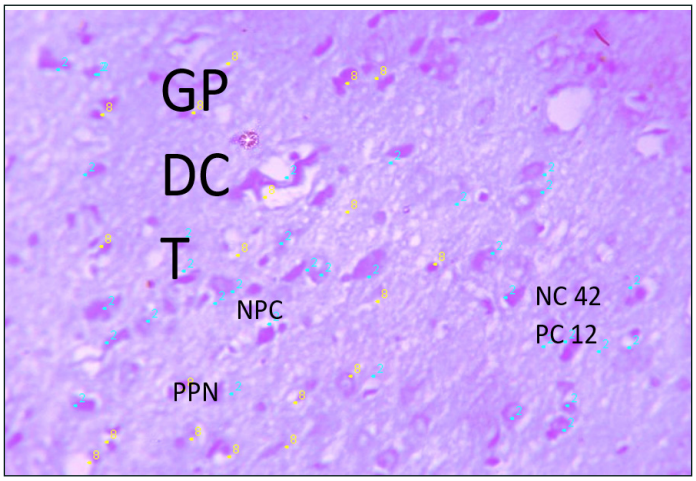


Plate 4: Photomicrograph of DCT section of cerebral cortex administered vit B low dose (x100(x400)(H/E) shows active pyramidal neuron (PPN) in CA1 region, numbering normal pyramial neuron (NPN) 42 and mild pyknotic pyramaidal neuron (PPN) 12

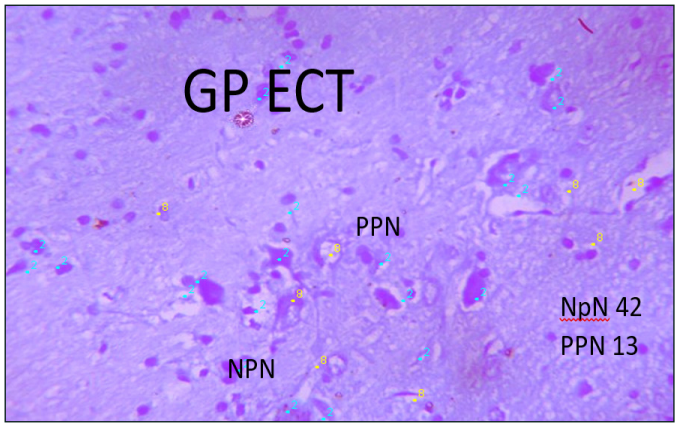


Plate 5: Photomicrograph of ECT section of cerebral cortex administered vit B high dose (x100(x400)(H/E) shows active pyramidal neuron (PPN) in CA1 region, numbering normal pyramial neuron (NPN) 42 and mild pyknotic pyramaidal neuron (PPN) 13

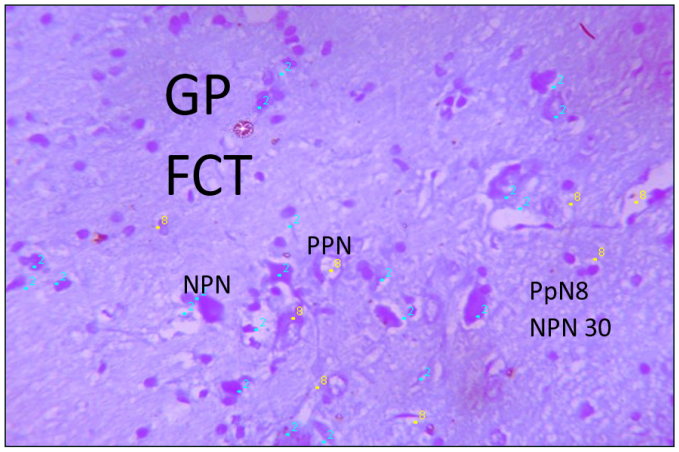


Plate 6: Photomicrograph of FCT section of cerebral cortex indused with treated metha and treated vit B low dose (x100(x400)(H/E) shows moderate regeneration with active pyramidal neuron (PPN) in CA1 region, numbering normal pyramial neuron (NPN) 30 and mild pyknotic pyramaidal neuron (PPN) 8

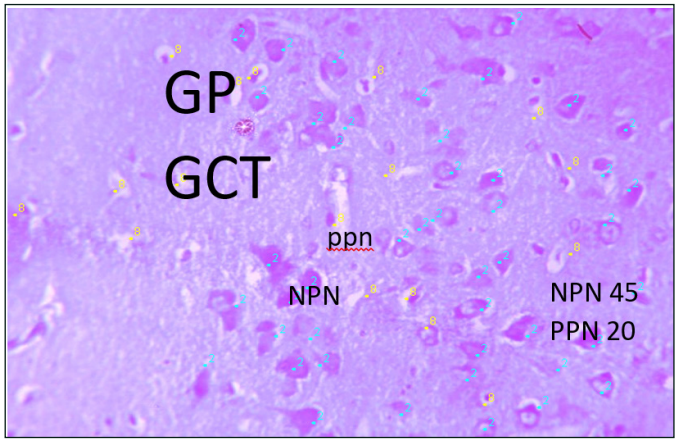


Plate 7: Photomicrograph of GCT section of cerebral cortex indused with high dose metha and treated vit B high dose (x100(x400)(H/E) shows moderate regeneration with active pyramidal neuron (PPN) in CA1 region, numbering normal pyramial neuron (NPN) 45 and mild pyknotic pyramaidal neuron (PPN) 20

Discussion

Observation showed that at the end of administration Group A (Control) which was given feed and water only showed a significant increase in weight, suggesting a proper feeding pattern.

Elevated malondialdehyde (MDA) levels observed in Groups B (2 mg/kg methamphetamine) and C (10 mg/kg methamphetamine) confirmed that methamphetamine exposure induces oxidative stress in the brain, consistent with previous findings that link methamphetamine to neuronal oxidative damage and lipid peroxidation (Johnson & Brown, 2020). These same groups also showed a significant reduction in body weight, further supporting the systemic toxicity of methamphetamine, which aligns with reports of weight loss and metabolic disruption in drug-intoxicated animals (Smith & Doe, 2021). Conversely, Groups D (50 mg/kg Vitamin B complex) and E (100 mg/kg Vitamin B complex) demonstrated significant weight gain and lower oxidative stress indices, indicating the beneficial role of Vitamin B complex in supporting metabolic and neuronal stability, in agreement with studies highlighting its antioxidant and restorative effects (Doe, 2009; Johnson & Brown, 2020). Furthermore, co-administration in Groups F (2 mg/kg methamphetamine + 50 mg/kg Vitamin B complex) and G (10 mg/kg methamphetamine + 100 mg/kg Vitamin B complex) ameliorated methamphetamine-induced toxicity, as reflected by significant weight gain and improved antioxidant status relative to Groups B and C, reinforcing evidence that Vitamin B supplementation mitigates oxidative damage and promotes recovery even under drug-induced neurotoxic stress (Smith & Doe, 2021).

Increased escape latency observed in Groups B (2 mg/kg methamphetamine) and C (10 mg/kg methamphetamine) indicates impaired spatial memory, learning and consciousness, confirming that methamphetamine disrupts cognitive function. In contrast, Groups D (50 mg/kg Vitamin B complex)

and E (100 mg/kg Vitamin B complex) demonstrated reduced escape latency, suggesting that Vitamin B complex alone may enhance cognitive performance. Moreover, the co-treatment groups, F (2 mg/kg methamphetamine + 50 mg/kg Vitamin B complex) and G (10 mg/kg methamphetamine + 100 mg/kg Vitamin B complex), showed significantly improved escape latency compared to Groups B and C, indicating that Vitamin B complex supplementation attenuates methamphetamine-induced impairments in spatial learning and memory. These findings align with evidence that B-vitamin supplementation supports neuronal function and exerts neuroprotective effects in conditions of drug-induced neurotoxicity (Johnson & Brown, 2020).

Photomicrography of the control group revealed distinct and moderately active granular cells, which aligns with the expected baseline cerebral cortex morphology (Bancroft & Gamble, 2008). This provides a clear histological benchmark against which experimental groups could be compared. In Groups B and C photomicrographs demonstrated moderate neuronal degeneration characterized by karyolysis, dissolution of nuclear material, and granular cell hyperplasia. These alterations indicate methamphetamine-induced neuronal toxicity, confirming that both low and high doses of methamphetamine negatively affect neural tissue integrity. The presence of granular cell hyperplasia may represent a compensatory mechanism in response to neuronal loss, as reported in earlier studies describing hyperplasia as an attempt by the brain to restore balance following toxic injury (Lillie, 1965). This observation is consistent with findings by Cadet & Krasnova (2009) and Rothman & Baumann (2003), who established that methamphetamine exposure, even at relatively low doses, leads to neuronal cell death, structural degeneration, and long-lasting neurotoxic effects.

In contrast, Groups D and E, which received 50 mg/kg and high-dose 100 mg/kg

Vitamin B complex respectively, showed only mild dissolution of neuronal cells and preservation of granular cells, suggesting that Vitamin B complex supplementation confers neuroprotection. The presence of intact granular cells in these groups highlights the potential of Vitamin B complex to stabilize neuronal membranes, enhance metabolic efficiency, and reduce oxidative burden within brain tissues. These findings align with Kennedy (2016), who emphasized the role of B vitamins in promoting neurological resilience, and Ninković & Bjugn (2011), who documented the restorative influence of B-complex vitamins on neuronal morphology in stressed models. The apparent dose-response effect further strengthens the case for Vitamin B complex, as higher doses (Group E) offered greater cellular protection than lower doses (Group D), which mirrors reports that optimal vitamin dosing enhances antioxidant and anti-inflammatory defenses.

Groups F and G, which received combined treatments of methamphetamine and Vitamin B complex, presented further evidence of this ameliorative effect. Group F (2 mg/kg methamphetamine + 50 mg/kg Vitamin B complex) demonstrated mild dissolution of neuronal.

Cells alongside active granular cells, which represents a marked improvement compared to methamphetamine-only groups. This suggests that Vitamin B complex supplementation, even at low doses, can mitigate the severity of methamphetamine-induced damage and restore some level of cellular activity. Similarly, Group G (10 mg/kg methamphetamine + 100 mg/kg Vitamin B complex) exhibited moderate healing with preserved cellular activity, indicating that high-dose Vitamin B complex is capable of significantly ameliorating methamphetamine-induced neurotoxicity, even under high toxic exposure. These findings corroborate the work of Stöver& Schlaepfer (2011) and de Oliveira & da Silva (2017), who emphasized the neuroprotective efficacy of B vitamins in mitigating neurochemical imbalances and oxidative stress-induced cell death.

Overall, the histological findings provide compelling evidence that methamphetamine intoxication exerts a dose-dependent neurodegenerative effect on the hippocampus and prefrontal cortex, while Vitamin B complex supplementation demonstrates a strong neuroprotective role by preserving cellular integrity and reducing neuronal dissolution. This protective effect appears to be both dose-dependent and synergistic, as higher doses of Vitamin B complex confer stronger protective outcomes, particularly when combined with methamphetamine exposure. The implications of these findings extend beyond

experimental models, as they suggest that Vitamin B complex may hold promise as a supportive therapy in mitigating methamphetamine-induced neuronal injury, thereby contributing to strategies aimed at preserving cognitive and emotional functions often impaired in drug abuse conditions.

Conclusion

The findings of this study demonstrate that methamphetamine exposure exerts dose-dependent neurotoxic effects, including weight loss, oxidative stress, cognitive impairments, and histopathological damage in the cerebral cortex of adult male Wistar rats. Elevated malondialdehyde levels confirmed increased oxidative stress, while reduced antioxidant markers further highlighted the imbalance in redox status. Behavioural deficits observed in methamphetamine-intoxicated groups were consistent with neuronal damage, supporting its role in impairing spatial learning and locomotor activity.

Conversely, supplementation with Vitamin B complex exerted protective effects across multiple parameters. Rats treated with Vitamin B complex showed improved body and brain weight indices, reduced oxidative stress, enhanced behavioural performance, and preserved neuronal architecture. These protective effects were dose-dependent, with higher doses offering greater amelioration against methamphetamine-induced damage. Notably, the combination of methamphetamine with high-dose Vitamin B complex demonstrated significant mitigation of neuronal injury, suggesting a potential therapeutic role of Vitamin B complex in combating drug-induced neurotoxicity.

Overall, the study provides compelling evidence that Vitamin B complex supplementation can reduce methamphetamine-induced neurotoxic damage, supporting its neuroprotective, antioxidant, and cognitive-enhancing properties.

Consent: Not applicable

Ethical Approval

Approval for this study was obtained from the Nnamdi Azikiwe University- Animal Research Ethics Committee with the approval number, NAU/AREC/2025/0086. This was carried out in strict compliance with "National Institutes of Health Guide for the Care and Use of Laboratory Animals" (NRC 2011).

Disclaimer (Artificial Intelligence)

Authors hereby declare that no generative AI technologies such as large language models (COPLOT, ChatGPT, etc) and text-image –generators have been used during writing or editing of this manuscript

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Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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