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THE EFFECTS OF THE CO-ADMINISTRATION OF METHAMPHETAMINE AND VITAMIN C ON THE AMYGDALA OF ADULT MALE WISTAR RATS

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ABSTRACT

Much attention has been drawn to the toxic effect of meth on humans. However, it is not clear whether or not the toxicity is related to some aspects of the brain like the amygdala, hence the present study is geared towards not only unraveling the toxic effect of meth on the amygdala but also investigating the protective effect of vitamin C against meth induced neurotoxicity. Thirty adult male wistar rats (185-220g) were divided into seven groups. Group A was given feed and water as the control; groups B and C were administered low and high dose of methamphetamine respectively; groups D and E were administered low and high dose of vitamin C respectively; group F was co-administered 5mg/kg body weight of meth and 300mg/kg body weight of vitamin, while group G was co-administered 10mg/kg body weight of meth and 600mg/kg body weight of vitamin C. All treatments were given orally daily for twenty one days. Twenty four hours after the last administration, the animals were anaesthetized under chloroform vapor and dissected, and the brain tissues were harvested through occipito-frontal incision and weighed. Some brain tissues were fixed in phosphate buffered formalin for oxidative stress analysis while others were fixed in 10% neutral buffered formalin for histological studies. The serum levels of malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD) in groups B, C, F and G differed significantly when compared to the control group. Histological observation showed that following administration of vitamin C, there was moderate increase in the number of active granular cells in the amygdala while following the administration of vitamin C to meth induced neurotoxicity, there was moderate regeneration with mild pyknotic granular cells present in the amygdala. The result of this study shows that slight adverse alterations to biomarkers of oxidative stress are associated with the co-use of methamphetamine and vitamin C and no histopathological lesions were found in the amygdala of adult male wistar rats. The findings of this study suggest that vitamin C administered to individuals exposed to methamphetamine could provide some protection against meth toxicity and perhaps ameliorate the effects of meth toxicity on the amygdala.

BACKGROUND OF THE STUDY

Methamphetamine is derived from amphetamine, which was broadly prescribed in the 1950s and 1960s as a medication for depression and obesity. Methamphetamine, called meth, crystal, or speed, is a central nervous system stimulant that can be injected, smoked, snorted, or ingested orally; prolonged use at high levels results in dependence (Douglas *et al*., 2000). The use of methamphetamine has reached epidemic proportions in major parts of the western and Midwestern US. Due to the localized nature of methamphetamine usage, there is conjecture that it might constitute a short-term issue rather than a prolonged one (Richard *et al*., 2001). Short and long term health effects of methamphetamine use include stroke, shaking, stomach cramps, insomnia, paranoia, irregular heartbeat, and structural changes to the brain. Among the substances of abuse in the United States, methamphetamine stands out as one of the less comprehensively studied drugs, possibly owing to its entirely synthetic composition and its relatively lower prominence at the national level when contrasted with

marijuana and cocaine (Hunt *et al*., 2006).

The relative ease with which the primary ingredients of methamphetamine can be acquired and then converted into the final product has led to the widespread existence of numerous ”mom-and-pop” laboratories, although larger criminal “super lab” organizations in Mexico, Canada and the United States continue to supply a large proportion of high-purity drug (Nordahl *et al*., 2003). The production of methamphetamine involves a simple one-step procedure, achieved by reducing ephedrine or pseudoephedrine and these ingredients are readily accessible in over-the-counter allergy medications in North America, and detailed instructions for the process of synthesis can be found online (Sulzer *et al*., 2005). Multiple preclinical and clinical investigations indicate that exposure to methamphetamine leads to substantial neural harm, correlating with cognitive decline. Currently, the available treatment of methamphetamine-induced psychosis and addiction are restricted, necessitating additional clinical trials.

Based on recent news coverage, there is a growing epidemic of crystal methamphetamine utilization or usage among young individuals in Eastern Nigeria. Eastern Nigeria consists of five states (Anambra, Imo, Abia, Enugu, and Ebonyi) and the main ethnic group or language of this region is Igbo. The popular name for methamphetamine is Mkpulummiri which is the Igbo name for ice in Eastern Nigeria (Declan, 2021). From October 2021 to December 2021, various media sources presented the utilization of crystal meth in different ways, proposing a multitude of factors that might be contributing to its use (Njoku *et al*., 2021). Every day, viral videos originating from different communities in the South-East depict individuals affected by Mkpulummiri, showcasing their associated unusual behaviors. Some of them were reported to have killed their parents, siblings or burnt their houses under the influence of the drug (Okoli *et al*., 2021). Due to the significant penalties and associated risks linked with trafficking cocaine and heroin, drug traffickers began establishing methamphetamine production facilities in West Africa in 2010 (UNODC, 2013). The most recent World Drug Report provides additional support to the idea that although methamphetamine production is declining in Asia and the Americas, the count of dismantled methamphetamine laboratories in Africa actually rose between 2010 and 2019 (UNODC, 2021). It is logical to deduce that the uptick in methamphetamine usage in the Eastern region and neighboring states of Nigeria might be attributed to the local laboratories, especially those established by drug traffickers. Mainly due to the availability of drugs, there is an encouragement of consumption and related negative consequences (Dumbili, 2020a).

Humans lack the necessary enzyme in the biosynthetic pathway, leading to the inability to synthesize the vital nutrient known as vitamin C (Nishikimi *et al*., 1994). The deficiency of this essential nutrient result in a potentially fatal disease called scurvy (Sauberlich *et al*., 1997). Scurvy is characterized by weakening of collagenous structures, resulting in poor wound healing, and impaired immunity (Hemila, 2017). Individuals with scurvy are highly susceptible to potentially fatal infections such as pneumonia (Hemila, 2017). The immune-modulating effects of vitamin C might arise from its various activities. It is a highly effective antioxidant, due to its ability to readily donate electrons, thus protecting important biomolecules like proteins, lipids, carbohydrates, and nucleic acids from damage by oxidants generated during normal cell metabolism and through exposure to toxins and pollutants like cigarette smoke (Carr and Frei, 1999). The skin contains millimolar concentrations of vitamin C, with higher levels found in the epidermis than the dermis (McArdle *et al*., 2002; Rhie *et al*., 2001; Shindo *et al*., 1994). In early investigations, vitamin C was recognized as a vital regulator in collagen production, with the active form, ascorbic acid, demonstrating strong antioxidant properties. The significant role of ascorbic acid (AA) as a cofactor for numerous prolyl and lysyl hydroxylases, integral to the maturation of collagen, has been firmly established.

The amygdala is an almond-shaped brain region located in the anterior portion of the temporal lobe. The amygdala, which takes up about 0.3% of the volume of the human brain, occupies roughly 2.25cm^3 on each side. The amygdala is a complex region made up of at least 13 nuclei and cortical areas in the brain (Amaral *et al*., 1992). It is an essential component of the limbic system, a neural network responsible for regulating various facets of emotion and memory.

Methamphetamine abuse can cause unpleasant psychological effects such as agitation, hallucination, and psychosis (Cruickshank and Dyer, 2009). Studies in abstinent human meth users indicate damage to the dopaminergic and serotonergic systems, marked by decreases in the dopamine transporters, DAT and serotonin transporters, SERT (McCann *et al*., 1998; Volkow *et al*., 2001a, b; Sekine *et al*., 2006). However, vitamin C treatment has been reported to improve neuropathological alterations as well as memory impairments and the neurodegenerative changes in rats exposed to neurotoxic substances (Olajide *et al*., 2017; Sil *et al*., 2016). Hence there is need to investigate the ameliorative effects of vitamin C on the neurological effects caused by methamphetamine on the amygdala.

METHODS

Ethical Approval

Ethical approval was obtained from the ethical committee, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University.

Experimental Design and Protocols

Thirty (30) adult male wistar rats were procured from

Research Enterprise farms, University of Ibadan. Perspex cages were used to house 7 groups of animals for routine experiment. Each cage had wire gauze top for cross ventilation. The animals were allowed for a period of two weeks for acclimatization at the animal house of Anatomy Department, Nnamdi Azikiwe University, Nnewi Campus, before their weight was taken, under a controlled room temperature of about 25-28°C, relative humidity of about 60-80% and photo-periodicity of 12h day / 12h night. They were fed Ad Libitum with water and guinea feed pallets from Agro feed mill Nigeria Ltd. All the animals were treated in accordance with the approval of ethical committee, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, in compliance 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 (1985).

Collection of Experimental Substances

Methamphetamine was procured from the locals and authenticated at the Department of Industrial Chemistry, Nnamdi Azikiwe University while vitamin C was procured from Madobi Pharmaceutical Stores, Nnewi, Anambra State and they were administered to the experimental animals by oral route.

Acute Toxicity Test (LD_{50}) of Methamphetamine

The acute toxicity test of methamphetamine and vitamin C was carried out in the Department of Biochemistry, Faculty of Basic Medical Sciences, Abia State University, Uturu, Abia state, according to the method employed by Dietrich Lorke (1983). In this study, a total of 13 adult wistar rats were used. The test was carried out in two phases. In phase 1, nine rats were used and they were grouped into three groups of three animals each. Group 1 received 10mg/kg of methamphetamine and vitamin C, group 2 received 100mg/kg of meth and vitamin C while group 3 received 1000mg/kg of meth and vitamin C. The animals were observed over a period of 24hrs for mortality. From the result of phase 1, the second phase was carried out. In this phase, 4 wistar rats were used and they were grouped into four groups of one animal each. Group 1 received 200mg/kg of meth and vitamin C, group 2 received 300mg/kg of meth and vitamin C, group 3 received 400mg/kg of meth and vitamin C while group 4 received 500mg/kg of meth and vitamin C. The animals were also monitored over a period of another 24hrs for mortality.

Administered Dosages and Duration

Group	Substances Administered (Feed and Water Adlibitum for all Groups)	Duration (days)
A	Feed and water only *adlibitum*	21days
B	5mg/kg or 0.43ml of methamphetamine	21days
C	10mg/kg or 1ml of methamphetamine	21days
D	300mg/kg or 0.3ml of dissolved vitamin C	21days
E	600mg/kg or 0.64ml of dissolved vitamin C	21days
F	5mg/kg or 0.43ml of methamphetamine and 300mg/kg or 0.3ml of vitamin C	21 days
G	10mg/kg or 1ml of methamphetamine and 600mg/kg or 0.64ml of vitamin C	21 days

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 C 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.

Termination of Treatment

The behavioral changes were tested across the 7 groups of animals to identify the ones that have lost their motor function. Twenty-four hours after the last exposure, following the behavioral test, the animals were weighed, then anaesthetized under chloroform vapor, and the animal skull dissected by occipitofrontal incision. The brain tissues were harvested from the animals and weighed. The brain tissues of some of the animals were prepared for biochemical analysis through the process of homogenization while the remaining tissues of some of the animals were fixed in 10% Neutral Buffered Formalin for 48hrs and grossed to isolate the brain tissue of interest for histological investigations in the histology laboratory of Anatomy Department, Nnamdi Azikiwe University, Nnewi campus.

Behavioral Function Test

Accurate assessment of motor function in experimental rats induced with toxic substance is critical for proper interpretation of basal ganglia and their behaviors especially movement (Zou *et al*., 2015).

Morris Water Maze Test

This test 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).

Method of Morris Water Maze Test

Biochemical Analysis

The brain tissues were taken to Biochemistry Department for the analysis of Malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD).

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) will be investigated with the method described by Ohkawa *et al.,* 1979. One milliliter (1mL) of 10% chilled (w/v) trichloroacetic acid (TCA) will be 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) will be added to 1ml of supernatant and kept in a boiling water bath for 10-15mins. After cooling, 1ml of distilled water will be added to it and absorbance will be taken at 530nm. The results will be 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) will be added to 1ml of 10% homogenate. The suspension will be left for 30mins and centrifuged at 2500rpm for 15mins. 0.5ml of supernatant will be taken and 2.5ml of 5'5'-dithionitrobenzoic acid (DTNB) will be added. The suspension will be shaken thoroughly and read at 412nm. The results will be expressed as µmol/g tissue.

Enzymatic antioxidant: Superoxide dismutase

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

Tissue Processing: After weighing the organs, a small part of the Substantia Nigra tissues 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 prevent autolysis and putrefaction. After fixation the tissues were dehydrated to remove water and other substances. This was carried out in different percentages of alcohol 50%, 70% and 95% absolute. In each grade of alcohol, tissues were changed twice for two (2) hours, one (1) hour for each change. After dehydration, tissues were cleared in xylene for two (2) hours after which infiltration was done in molten paraffin wax at a temperature of 60oc for two (2) hours, each in two changes. When the paraffin wax cools, it sets as a hard block which allows for easy sectioning of the tissues. The tissue sections were produced by normal histochemical methods of dehydration, clearing, impregnation, embedding, sectioning and staining (with H&E). The micrographs of the relevant stained sections were subsequently taken with the aid of a light microscope.

Statistical Analysis: The data were analyzed using SPSS version 23. Values were represented as MEAN and SEM, Relative Organ weight (Brain) were analyzed using One way ANOVA, followed by Post Hoc LSD multiple comparison. Body weight was analyzed using Student dependent T-test. Values were considered significant at $P<0.05$.

RESULTS

Physical and Behavioral Observations

At the beginning of the experiment, all the animals were apparently healthy and agile. During the two weeks of acclimatization, their stool was normal and they adapted well to their environment. During the 21days of co-administration of methamphetamine and vitamin C, the following behaviors were observed:

a) Increased water intake in groups D and E.
b) Aggressiveness and restlessness in groups B, C, F and G.
c) Excessive grooming and rearing in groups B, C, F and G.
d) All groups had steady growth throughout the duration.
e) Decreased hyperactivity in groups F and G after day

10 of administration.

f) Reduced food intake in groups E, F and G.

g) Excessive drowsiness in groups D and E.

Table 1: Effect of Methamphetamine and Vitamin C on Mean Initial Body Weight and Final Body Weight in all Groups.

Groups	Weight (g)	Mean ± SEM	p-value
Group A	Initial Final	210.05 ± 0.8 235.33 ± 1.76	0.044
Group B	Initial Final	202.33 ± 0.88 196.67 ± 1.20	0.001
Group C	Initial Final	201.33 ± 0.88 192.00 ± 2.00	0.001
Group D	Initial Final	200.33 ± 0.88 209.00 ± 2.00	0.001
Group E	Initial Final	203.02 ± 0.13 211.05 ± 0.56	0.001
Group F	Initial Final	200.33 ± 0.67 195.67 ± 0.88	0.001
Group G	Initial Final	201.33 ± 0.88 194.67 ± 1.20	0.001

Data was analyzed using Student dependent T-test and values were considered significant at P<0.05.

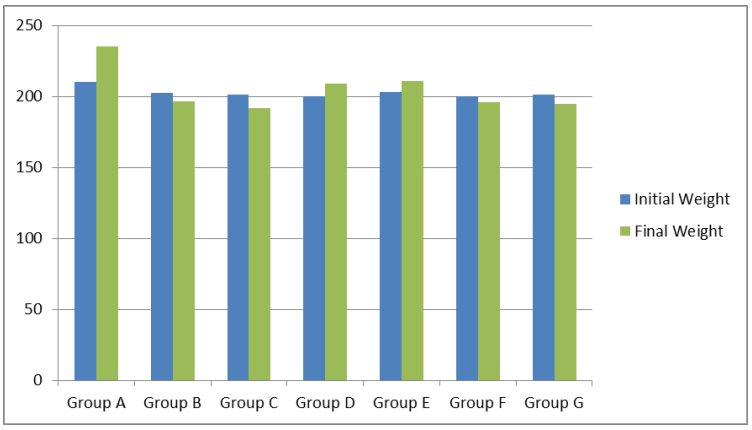

Fig. 1: Bar Chart Showing the Effect of Meth and Vitamin C on the Body Weights of Rats.

Result from the chart above shows that there was a significant increase in the body weight when the initial weight (210±0.8) was compared to the final weight (235.33±1.76) in group A. In group B, there was a significant decrease when the initial body weight (202.33±0.88) was compared to the final body weight (196.67±1.20). For group C, there was a significant decrease in the body weight when the initial weight (201.33±0.88) was compared to the final weight (192.00±2.00). For group D, there was a slight increase in the body weight when the initial weight (200.33±0.88) was compared to the final weight (209.00±2.00). For group E, there was a slight increase in body weight when the initial weight (203.02±0.13) was compared to the final weight (211.05±0.56). In group F, there was a significant decrease when the initial body weight (200.33±0.67) was compared to the final body weight (195.67±0.88). For group G, there was a significant decrease in the body weight when the initial weight (201.33±0.88) was compared to the final weight (194.67±1.20).

Table 2: Result of Morris Water Maze Test Conducted on Meth and Vitamin C induced rats.

	Groups	Mean ± SEM	p-value	f-value
Morris Water Maze Test-Escape latency (Seconds)	Group A	16.52 ± 2.93		6.369
	Group B	40.25 ± 3.84	0.044	
	Group C	43.50 ± 11.23	0.002	
	Group D	22.50 ± 5.23	0.001	
	Group E	24.35 ± 9.59	0.001	
	Group F	27.20 ± 4.54	0.341	
	Group G	40.25 ± 3.84	0.044	

Data were analyzed using One-way ANOVA, and values were considered significant at P<0.05.

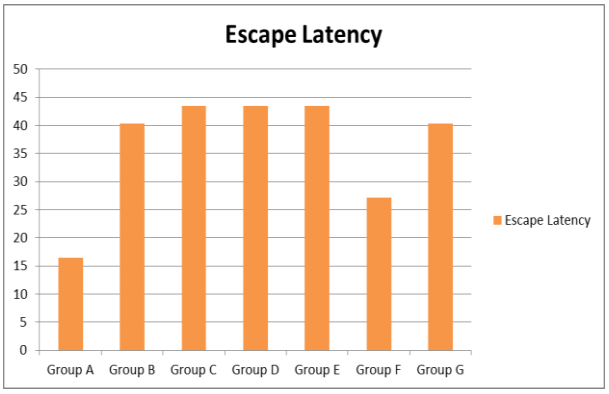

Fig. 2: Bar Chart showing Escape Latency of Rats from Morris Water Maze Test.

In the Morris Water Maze Test, the chart above shows that groups D (22.50±5.23) and E (24.35±9.59) had a significant difference when compared to groups B (40.25±3.84) and C (43.50±11.23). Groups B and C that received methamphetamine had a significant difference when compared to group A. Group F (27.20±4.54) had a significant difference while group G (40.25±3.84) had an insignificant difference when compared to group A.

Table 3: Oxidative Stress Biomarkers.

	Groups	Mean ± SEM	p-value	F-value
MDA	Group A	3.42 ± 0.01		
	Group B	3.64 ± 0.02	0.000	44.160
	Group C	3.72 ± 0.06	0.000	
	Group D	3.28 ± 0.06	0.000	
	Group E	3.24 ± 0.06	0.000	
	Group F	3.52 ± 0.01	0.000	
	Group G	3.64 ± 0.02	0.000	

	Groups	Mean ± SEM	p-value	F-value
GSH	Group A	1.44 ± 0.02		
	Group B	1.30 ± 0.03	0.031	14.246
	Group C	1.25 ± 0.02	0.002	
	Group D	1.25 ± 0.02	0.002	
	Group E	1.25 ± 0.02	0.002	
	Group F	1.40 ± 0.01	0.890	
	Group G	3.64 ± 0.02	0.031	

	Groups	Mean ± SEM	p-value	F-value
SOD	Group A	8.56 ± 0.02		
	Group B	7.54 ± 0.01	0.000	1336.758
	Group C	7.33 ± 0.03	0.000	
	Group D	7.33 ± 0.03	0.000	
	Group E	7.33 ± 0.03	0.000	
	Group F	7.80 ± 0.02	0.000	
	Group G	7.54 ± 0.01	0.000	

Data were analyzed using One-way ANOVA, followed by Post HOC Fisher's LSD multiple comparison, and data was considered significant at P<0.05

MDA= Malondialdehyde, GSH= gluthathione, SOD= superoxide dismutase

HISTOLOGICAL FINDINGS

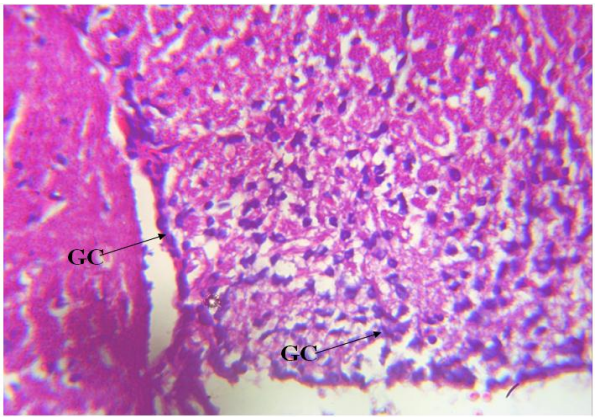

Plate 1: Photomicrograph of group A section of amygdala (x100 x400/ H&E) shows numerous aggregate of granular cells (GC) and other neuronal cells that are normal.

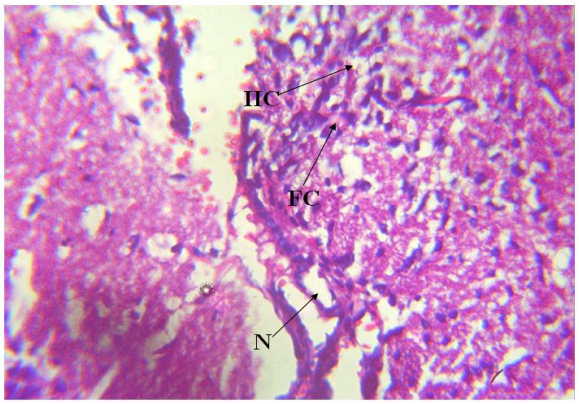

Plate 2: Photomicrograph of group B section of amygdala induced with 5mg/kg of methamphetamine only (x100 X400/(H/E) shows moderate to severe degeneration with necrosis (N), moderate fatty change (FC) and infiltration of inflammatory cells (IIC).

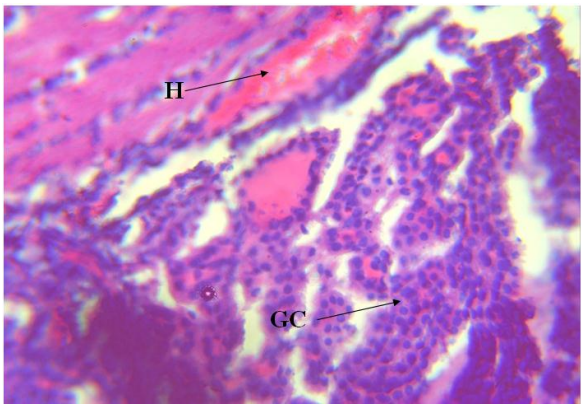

Plate 3: Photomicrograph of group C section of amygdala induced with 10mg/kg of methamphetamine only (x100 X400/ (H/E) shows severe degeneration with severe granular cell hyperplasia (GCH) and focal area of hemorrhage (H).

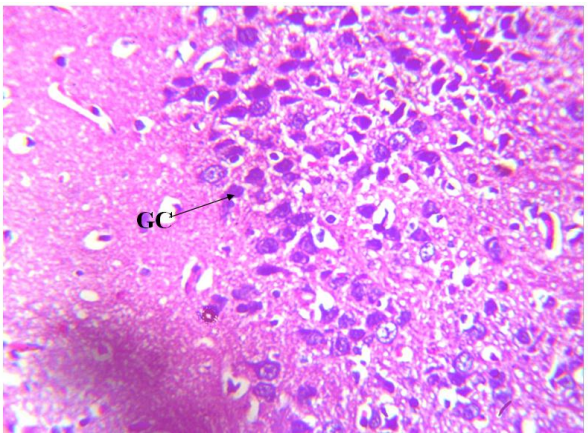

Plate 4: Photomicrograph of group D section of amygdala administered 300mg/kg of vitamin C (x100 x400/H&E) shows moderate increase in the number of active granular cells (GC).

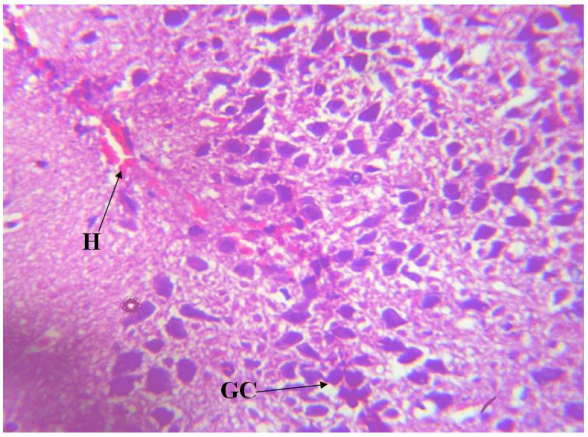

Plate 5: Photomicrograph of group E section of amygdala administered with 600mg/kg of vitamin C (x100 x400/H&E) shows moderate increase in the number of active granular cells (GC) and mild focal area of hemorrhage (H).

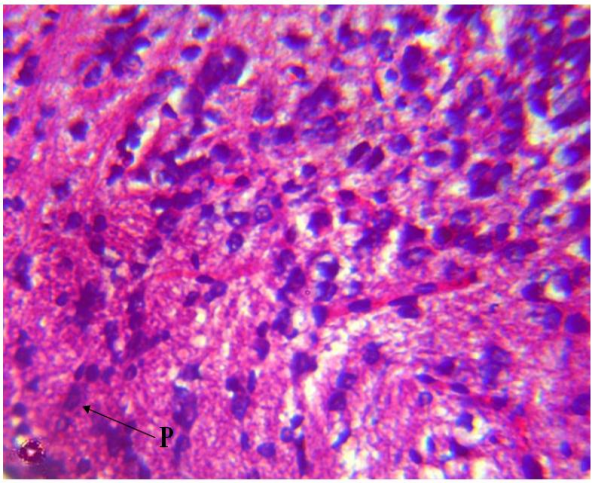

Plate 6: Photomicrograph of group F section of amygdala induced with 5mg/kg of meth and 300mg/kg of vitamin C (x100 x400/H&E) shows moderate regeneration with mild pyknotic (P) granular cells.

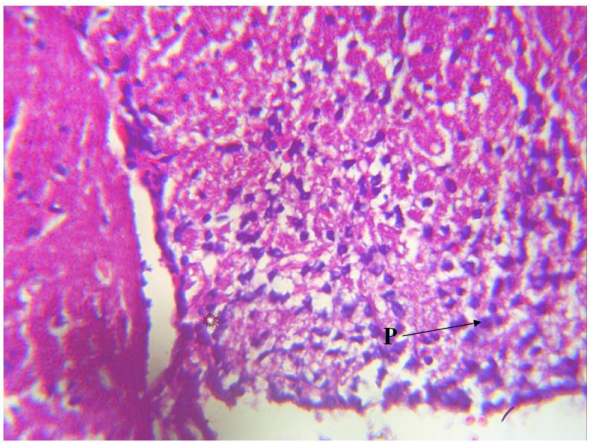

Plate 7: Photomicrograph of group G section of amygdala induced with 10mg/kg of meth and 600mg/kg of vitamin C (x100 x400/H&E) shows moderate regeneration with mild pyknotic (P) granular cells.

DISCUSSION

Methamphetamine, called meth, crystal, is a central nervous system stimulant that can be injected, smoked, snorted, or ingested orally; prolonged use at high levels results in dependence (Anglin *et al*., 2000). Short and long term health effects of methamphetamine use include stroke, insomnia, paranoia, irregular heartbeat, and structural changes to the brain (Perrochet *et al*., 2000). Vitamin C is a highly effective antioxidant, due to its ability to readily donate electrons, thus protecting important biomolecules like proteins, lipids, carbohydrates, and nucleic acids from damage by oxidants generated during normal cell metabolism and through exposure to toxins and pollutants like cigarette smoke (Carr and Frei, 1999).

This research work was carried out to ascertain possible effects of meth and vitamin C on the amygdala of adult male wistar rats and the result of this study reveals that the intake of these substances caused some behavioral and physical changes in adult male wistar rats.

On the physical and behavioral observations, it was observed that oral exposure to vitamin C only had no negative effect on the behavior of the animals while oral exposure to meth caused some characteristic behavioral changes in the animals. Observation of the body weight difference in groups reveals increase in body weight of animals in group A. This could be physiological as the only substance they were exposed to was water and feed. There was significant reduction in body weight of animals in groups B, C, F and G when compared with control. This is probably as a result of degeneration in adipose tissue of the animals. This result agrees with a previous study done by Hassan *et al*., (2016) who reported degenerative changes in adipose tissue together with inflammation and necrosis following meth use. The groups that were administered with only vitamin C showed a slight increase in body weight when compared to the control group. This is in line with the findings of

Ping-Chi *et al* who reported that while vitamin C plays a crucial role in maintaining normal health through its antioxidant and detoxification properties, it doesn't notably contribute to body weight gain on its own.

Results from Morris Water Maze test conducted showed significant differences in escape latency time in all other groups when compared to group A (control). In groups B, C, F and G, the escape latency time were significantly prolonged than in the control group. This could be due to the negative effects of meth in learning ability and memory retention. This is in line with a previous work of Schutová *et al*., (2009) who reported spatial learning and memory deficits associated with increased levels of anxiety and aggressive behavior in meth induced rats. Even though vitamin C is known to have neuroprotective effects, it could not prevent the deteriorative effects of meth on memory and spatial learning in rats.

Oxidative stress results from an imbalance between the oxidative and antioxidant systems of cells and tissues and is the result of excessive production of oxidative free radicals and related reactive oxygen species (ROS). Malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD) are the main biomarkers of oxidative stress. MDA is a byproduct formed from the lipid peroxidation reaction that provides information about the redox state (França *et al*., 2013), GSH is an important antioxidant that helps neutralize free radicals and peroxides, while SOD is an effective intracellular antioxidant enzyme which provides the first line of defense against the toxic effects of numerous ROS (Gill and Tuteja, 2010).

In the present study, there was significant increase in MDA level of animals in groups B and C and this could be due to the neurotoxic effects of meth. This is in line with Kristen *et al*., (2011) who reported that repeated meth administration results in increase in MDA-modified proteins in several regions of the brain. In groups F and G, there was a slight increase in MDA levels when compared to the control group. This result indicated that the methamphetamine did not bring about much neurocellular damage in the amygdala of animals in groups F and G. This could be attributed to the ameliorative effects and antioxidant properties of vitamin C. This agrees with Ya-Ni Huang *et al*., (2016) who reported the protective effect of vitamin C against meth toxicity through attenuation of ROS production, autophagy and apoptosis. There was also a significant decrease in GSH and SOD levels in groups B, C, F and G relative to the control group. Findings of Ramesh *et al.,* (2019) show that increased ROS level with chronic meth treatment causes severe depletion of GSH and SOD level. However, this study shows that treatment with vitamin C reduced the deteriorative effects of meth on GSH and SOD level in the amygdala of animals in groups F and G.

Histological findings of the present study indicated numerous aggregate of granular cells and normal neuronal cells of the amygdala in control group as seen in the photomicrograph of plate 1. The group D animals administered a low dose of vitamin C showed moderate increase in the number of active granular cells in the amygdala as seen in the photomicrograph of plate 2. This is in line with the findings of Pei *et al*., (2010) stating that vitamin C favored the generation of induced pluripotent stem cells. This result also agrees with Katterine *et al*., (2023) who reported that vitamin C induces the expression of stem cell-specific microRNA which promotes stem cell self renewal. Photomicrograph of group E animals in plate 3 administered with 600mg/kg of vitamin C also showed an increase in the number of granular cells, similar to those in group D.

Photomicrograph of animals in group B showed moderate to severe degeneration with necrosis, moderate fatty change and infiltration of inflammatory cells, while that of animals in group C showed severe degeneration with severe granular cell hyperplasia and focal area of hemorrhage. This result agrees with a previous study done by Hassan *et al*., (2016) who reported degenerative changes in adipose tissue together with inflammation and necrosis following meth use.

Animals in groups F and G co-administered with methamphetamine and vitamin C showed a moderate regeneration of mild pyknotic granular cells in photomicrographs of plate 6 and 7 respectively. This could be due to apoptotic effect of meth on cells. This agrees with Tulloch *et al*., (2011) who reported that exposure to a neurotoxic dose of meth induces stages of cell death in the striatum. However as seen in the photomicrographs, there was moderate regeneration of these pyknotic cells in the amygdala and this could be attributed to the regenerative function of ascorbic acid on dying cells.

The animals in groups B and C gives a particularly interesting observation about the dynamics of reactions to the presence of various substances in our systems. On administration of meth and vitamin C to the groups, physical and behavioral changes were observed; there was slight decrease in body weight which could be due to reduced food intake, and regeneration of pyknotic cells in the amygdala was observed histologically. By these observations, it is fair to conclude that administration of vitamin C may boost the tolerance capacity for meth induced toxicity.

Thus, the neuroprotective and neurodegenerative effect of vitamin C against methamphetamine induced amygdala recorded in the present study is attributed to its antioxidant properties.

CONCLUSION

The coadministration of methamphetamine and vitamin C induced slight alterations in oxidative stress biomarkers of serum levels of malondialdehyde (MDA),

glutathione (GSH) and superoxide dismutase (SOD) and no histopathological lesions was observed in amygdala tissues of the rats, though there were mild pyknotic granular cells. The findings of this study suggest that vitamin C administered to individuals exposed to methamphetamine could provide some protection against meth toxicity and perhaps ameliorate the effects of meth toxicity on the amygdala.

RECOMMENDATIONS

This study has opened a way for possible means of preventing damage to man tissues by meth. I recommend that other vitamins like alpha-tocopherol (vitamin E) should be administered to see if it is possible to demonstrate a possible counter effect on the amygdala. However, further work is needed particularly on the effect of these drugs on other organs like stomach, kidney etc.

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