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Vitamin C And E Protects Wistar Rats Brain (Substantia Nigra) From Rohypnol Induced Neurotoxicity

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Abstract: Rohypnol is a benzodiazepine medication that is medically prohibited in the United States but is lawfully prescribed in more than sixty other countries. The drug is used as a pre-anesthetic and to treat insomnia, but it causes Substantia Nigra function to be lost, which is what motivated this study to determine whether vitamin C and E can improve the effects of adult wistar rats' exposure to Substantia Nigra. Twenty-five adult male Wistar rats were randomly separated into five groups of five rats each (A, B, C, D, and E). Group A, the control group received distilled water. Groups B, C and D received 1mg/kg of Rohypnol, 1mg/kg of Rohypnol+100mg/kg of vitamin C, 1mg/kg of Rohypnol+100mg/kg of vitamin E and 1mg/kg of Rohypnol+100mg/kg of vitamin C and E of Rohypnol respectively. The animals were sacrificed after three weeks of administration and the substantia Nigra were harvested and fixed in 10% formal saline for histological processing and studies. The results obtained in this study following the administration of Vitamin C & E on substantia Nigra exposed rohypnol shows a significant increase in the test group when comparing to the other group. The crucial role of Vitamin C and E in neuronal maturation and functions, neurotransmitter action as well as responses to oxidative stress is well supported by the evidences presented in this study. In conclusion This study have shown that good antioxidants such as vitamin C and E are very much effective in reducing oxidative stress and thus, have great antioxidant ameliorative properties to the Rophynol induced substantia nigra of the wistar rats.

Keywords: Flunitrazepam Vitamin C and E (Rohypnol), Substantia Nigra, Histoarchitecture, Benzodiazepine.

Cite this article:

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Introduction

Under the commercial name Rohypnol, flunitrazepam is a medication that belongs to the benzodiazepene family. It is used in Mexico, Europe, South America, and Asia as a pre-anesthetic medication and as a sleeping tablet. Rohypnol is a tranquilizer similar to Valium, but ten times as effective. Roofie is the name of the street. In the US, prescribing it is against the law. In Nigeria, benzodiazepine addiction is still rather prevalent among young people. (Olaniyan et al., 2017). Gamma-aminobutyric acid (GABA), a neurotransmitter that reduces brain activity, is enhanced by rohypnol. Rohypnol has sedative, hypnotic, anxiolytic (anti-anxiety), muscle-relaxant, and anticonvulsant properties via increasing the inhibitory actions of GABA by binding to certain receptors in the brain. Its potency and quick start of action make it

useful for lowering anxiety and promoting sleep. (Rang et al 2016, katzung and Trevor 2017, Goodman et al 2018, Nutt et al 2012)

As stated by Drug Facts. Rohypnol is given for the short-term treatment of severe insomnia and as a pre-anesthetic prescription to relieve anxiety and produce calm before to surgical procedures, according to the National Institute on Drug Abuse. Club drugs (GHB, Ketamine, and Rohypnol). Due to its sedative qualities, it is beneficial for individuals who need help falling asleep quickly or managing anxiety. Although rohypnol has medical benefits, it is often abused for recreational purposes. (Szaflarski and Sirven 2017) The drug is often sought after for its ability to produce feelings of euphoria, relaxation, and disinhibition. (Buchanan et al,202) However, when paired with alcohol or other CNS depressants, its strong sedative effects can cause respiratory depression, memory loss, and loss of motor coordination.. (Bohmwald et al, 2021)

The use of Rohypnol in drug-facilitated sexual assault (DFSA) is among its most concerning misuses. Rohypnol has been used to incapacitate people due to its potent sedative and amnesic effects, leaving them open to sexual assault without permission. (Thomas and Kopel 2023).

Rohypnol abuse can result in a number of negative side effects, such as respiratory depression, drowsiness, confusion, dizziness, slurred speech, and impaired judgment. Long-term abuse can lead to tolerance, physical dependence, withdrawal symptoms when stopping, and possibly even a potentially fatal overdose. (Akagi and Tasaka, 1991)

According to Baldwin (Baldwin 2022) Despite the availability and acceptability of other pharmacological and psychological treatments, benzodiazepines are nevertheless often used to treat individuals with anxiety disorders or sleeplessness. Many patients will have side effects during their course of treatment, and they will be quite distressed when the dosage is lowered and terminated. The management of benzodiazepine withdrawal entails steps to stop dependence from forming, close monitoring of underlying medical conditions, consolidation of medications and gradual dosage reduction, occasional prescription of concomitant medication, prevention of relapse with ongoing support to address psychosocial stressors, and accompanying psychological interventions. (Baldwin 2022), In addition to investigating the effectiveness of alternative pharmacological classes in managing benzodiazepine withdrawal, more study is required to enhance tactics for avoiding dependency and aiding withdrawal.

The possibility of vitamins C and E to mitigate neurodegenerative illnesses, such as Parkinson's disease and Alzheimer's, has been suggested. The co-administration of vitamins C and E did not significantly improve neuroprotection over single vitamin administration in a study on reserpine-induced oxidative stress in mice. The literature data that is currently available suggests that vitamin C and E deficiency is very common in patients with depressive disorders. Gariballa 169

In animal models of neurodegenerative disorders, vitamin E treatment has demonstrated beneficial effects on indicators of neurodegeneration and neuroprotection. (Da Cunha Germano et al, 2023). Overall, the effects of co-administration of vitamin C and E on neurodegenerative disorders are still not fully understood and require further research (Danboyi et al 2019), However, the effects of vitamin E supplementation in clinical trials are inconsistent, and genetic variants can influence its overall effect (Regner-Nelke et al 2021)

Dopamine is produced primarily in the substantia nigra, an area of the brain that is important for numerous central nervous system activities, including movement control, cognitive executive skills, and emotional limbic activity. (Murray et al, 2019), The substantia nigra gradually degenerates over the course of a lifetime or throughout old age, resulting in the majority of types of Parkinson disease; however, the source of this degeneration is unclear. (Murray et al 2019), The purpose of this research is to determine the underlying processes of vitamin C and E attenuation as well as rohypnol.

Materials and Methods.

Location and Duration of the Study

This scientific study was conducted at 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 14days after which the test substances was administered for 28days; the entire experiment lasted for six weeks

Ethical Approval

The Nnamdi Azikiwe University, Awka Nnewi Campus, Faculty of Basic Medical Sciences ethical committee provided the ethical permission.

Experimental Animals: Twenty five (25) male albino rats weighing 150-200g were procured from a laboratory at Nnamdi Azikiwe University Nnewi campus and housed at the animal house of the Department of Anatomy, College of Health Sciences Nnamdi Azikiwe University Nnewi, Anambra State. The rats were housed in plastic cages with iron nets in conventional conditions (controlled room temperature of 25-28 ℃, relative humidity of 60-80%, and photoperiodicity of 12 hours day and night). The rats were adequately fed with standard grower mesh manufactured by Premier Feed Mills Co. Limited (a subsidiary of Flour Mills Nigeria Plc). The animals were divided into four groups: Group A represents the control group, and Groups B, C, and D represent the test group. All rats were weighed before medication and then weekly (once a week) using Melter's Electronic weighing balance model PB303 (manufactured by Monobloc in Switzerland).

Procurement of Drug: Rohypnol was produced and marketed by SWISS Pharma Nigeria LTD. No 5, Dopemu Road, Agege, Lagos state. Under the license of Global Healthcare Ltd, Basel Switzerland.

Experimental Design: The animals were divided into four groups at random, with five animals in each group. For 28 days, the test animals received varying dosages of Rohypnol (1 mg/kg of Rohypnol, 1 mg/kg of rohypnol +100 mg/kg vitamin C, 1 mg/kg of Rohypnol+ 100 mg/kg of vitamin E and 1 mg/kg of Rohypnol+ 100 mg/kg of vitaminC and E). Group A, the control group, on the other hand, was given simply distilled water.

S/N	GROUP	WATER+RAT FEED	DURATION
1	A (Control)		28days
2	B	1mg/kg of Rohypnol	28days
3	C	1mg/kg of Rohypnol+100mg/kg of Vitamin C	28days
4	D	1mg/kg of Rohypnol +100mg/kg of Vitamin E	28days
5	E	1mg/kg of Rohypnol +100mg/kg of Vitamin C and E	28days

Mode of exposure:

The experimental animals were exposed by oral administration of rohypnol solution in water and vitamin C and E solution in water, respectively, through a cannula at room temperature based on the dosage for each group.

Termination of treatment:

To establish whether animals had lost their motor function, behavioural changes were examined in each of the five animal groups. The animals were weighed, anaesthetized with chloroform vapour 24 hours after the last exposure, and the skull was dissected via occipito-frontal incision following the behavioural test. The animals' brain tissues were extracted and weighed. While the remaining tissues of some of the animals were fixed in 10% Neutral Buffered Formalin for 48 hours and grossed to isolate the brain tissue of interest for histological investigations in the anatomy department's histology laboratory at Nnamdi Azikiwe University, Nnewi campus, the brain tissues of some of the animals were homogenised in preparation for biochemical analysis.

Behavioral Function Test

Rats rely on cues to detect food sources, recognize social and mating partners, and avoid predators, which require locomotion (Zou *et al*., 2015). Accurate assessment of motor function in experimental rats exposed to toxic substance is critical for proper interpretation of basal ganglia and their behaviors especially movement.

Wire Suspension Test

The wire suspension test was used to compare the motor function of different animal groupings. The animals were suspended by their forepaws from a 2mm-diameter metal bar elevated 30cm above a soft surface. The puppies' time to lose their grasp and fall on the soft surface was measured. When the animal could hang on the bar for 30 seconds, it was thought that it had fully mastered the reflex.

Method of Wire Suspension Test

A 55cm wide 2-mm thick metallic wire was secured to two vertical stands. The wire was tightly attached to the frame to avoid vibration or unwanted displacement of the wire while handling the animals, since these unwanted effects would interfere with the animal's performance. The wire was maintained 35cm above a layer of bedding material to prevent injury to the animal when it falls down. A fixed limit was used and a hanging time of 6minutes. The start position of the rat may also vary; with either two or four limbs.

1. The video camera was turned on and the rat, handled by the tail, was allowed to grasp the middle of the wire with its fore limbs and is gently lowered so that its hind paws grasped the wire a few cm apart from the fore paws.

2. The rat was then gently accompanied while it turns upside-down along the axis of the wire.

3. The tail was released while the rat was still grasping the wire with its four paws. Upon release, the timing started.

4. The time until the rat completely released its grasp and falls down was recorded.

5. The rat was given three trials per session, with 30-sec recovery period between trials.

7. The data for each group was analyzed using ANOVA to determine significant average hanging time of the three trials per session given in between each test.

Biochemical Analysis

The brain tissues were taken to Biochemistry Department, Nnamdi Azikiwe University, Awka for the analysis of Malondialdehyde (MDA), specific antioxidant capacities (GSH, SOD, GPx).

Indication:

Because MDA reacts well with thiobarbituric acid, it has been utilized extensively for many years as a simple biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids. One of the end products of polyunsaturated fatty acid peroxidation in the cells is malondialdehyde (MDA). Overproduction of MDA is caused by a rise in free radicals. A typical indicator of oxidative stress and the antioxidant status in cancer patients is the quantity of malondialdehyde. Antioxidants are predicted to decrease in brain illness conditions when the MDA level rises. An indicator of the antioxidant status of biological materials, antioxidant can also measure the antioxidant response to the free radicals generated in a particular disease. The two substrates for energy metabolism in the brain in the normal in vivo state are glucose and oxygen, but glucose is the sole important substrate.

Homogenization

Each organ weighs one gram, which is then combined with ten milliliters of 0.9% normal saline and homogenized at room temperature using a homogenizer. Every sample was then centrifuged for 20 minutes at room temperature at 3000 rpm. After being separated, the supernatant was kept for later examination at 2 degrees Celsius in a refrigerator.

Lipid peroxidation

Lipid peroxidation (LPO) will be examined using the method proposed by Ohkawa et al. 1979. One millilitre (1mL) of 10% cooled (w/v) trichloroacetic acid (TCA) will be mixed with one millilitre (1mL) of 10% homogenate, incubated at 37°C for 10

minutes, then centrifuged at 2,500 rpm for 15 minutes at room temperature. One millilitre (1mL) of 0.67% thiobarbituric acid (TBA) will be added to 1 mL of supernatant and left in a boiling water bath for 10-15 minutes. After cooling, add 1 cc of pure water and measure absorbance at 530 nm. The data will be presented as nmol MDA/h/g tissue.

Non-enzymatic antioxidant: Reduced glutathione

Glutathione (GSH) will be examined using the Ellman (1959) approach. One millilitre of 5% TCA (w/v) will be mixed with 1 ml of 10% homogenate. The suspension will be left for 30 minutes before centrifugation at 2,500 rpm for 15 minutes. 0.5 mL of supernatant will be collected, and 2.5 mL of 5'5'-dithionitrobenzoic acid (DTNB) will be added. The suspension will be properly shaken and measured at 412 nm. Results will be presented in µmol/g tissue.

Enzymatic antioxidants

Superoxide dismutase

Superoxide dismutase (SOD) will be studied using the approach outlined by Kakkar et al. (1984). Add 650 µl of sodium pyrophosphate buffer, 50 µl of brain supernatant fraction, 50 µl of phenazine methosulfate (PMS), 150 µl of nitroblue tetrazolium (NBT), and 100 µl of nicotinamide adenine dinucleotide phosphate (NADPH) to the mixture and thoroughly vortex. To stop the process, add 500 µl of glacial acetic acid after incubating for 90 seconds. Two millilitres of n-butanol will be added and vortexed completely. It will be maintained at room temperature for ten minutes. The absorbance will be measured at 560 nm. The data will be represented as µmol/min/mg protein.

Glutathione peroxidase

Glutathione peroxidase (GPx) will be investigated with the method described by Rotruck *et al*. (1973). 0.4 ml tris-HCl buffer (pH 7.5, 0.1 M), 0.2 ml GSH, 0.1 ml sodium azide, 0.1 ml distilled water, 0.1 ml H 2 O 2 and 0.1 ml of enzyme (supernatant fraction) will be mixed well and incubated at 37°C for 15 min. After incubation, 0.5 ml TCA will be added and centrifuged. 0.5 ml of supernatant will be taken, and 2 ml Na2HPO4 2H2O and 0.5 ml Ellman's reagent was added. Absorbance will be noted at 420 nm. The results will be expressed as nmol/min/mg protein.

Organ Collection: The rats were given graduated doses of Rohypnol for 28 days before being slaughtered. After being harvested and kept in normal saline to preserve their physiological state, the Substantia Nigra were weighed and fixed in 10% formal saline in preparation for histological processing.

Tissue Processing: Following the organs' weight measurement, a tiny portion of the Substantia Nigra tissues was removed and fixed right away in 10% formal saline to maintain the various cell components in their proper microanatomical positions and to stop autolysis and putrefaction. The tissues were dehydrated after fixation in order to eliminate water and other materials. This was done with varying absolute alcohol levels of 50%, 70%, and 95%. Tissues were replaced twice over the course of two hours, one hour for each grade of alcohol. Following dehydration, tissues were cleaned in xylene for two hours, and then they were infiltrated twice a day, for a total of two hours, in molten paraffin wax at 60 degrees Celsius. The paraffin wax forms into a solid block when it cools, making tissue sectioning simple. The typical histochemical procedures of dehydration, clearing, impregnation, embedding, sectioning, and staining (using H&E) were used to create the tissue sections. A light microscope was then used to take the micrographs of the pertinent stained sections.

Statistical Analysis: The analysis of the data was done with SPSS version 23. The data were provided as Mean and Standard Error (SEM). One-way ANOVA was used to examine the relative organ weight (brain), and Post Hoc LSD multiple comparison was then performed. Student dependent T-test analysis was used to determine body weight. When a value was $P<0.05$, it was deemed significant.

Results

Oxidative Stress Markers and Antioxidants Activity

Table 1: Effect of Vitamin C and E on motor function using wire test following Rophynol toxicity

	Day 1 (Seconds)	Day 2 (Seconds)	Day 3 (Seconds)
	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group A (control)	187.00±6.00	193.00±10.70	241.00±9.00
Group B (1mg/kg of Rophynol)	56.50±9.50*	169.00±6.00*	175.50±8.50
Group C (1mg/kg of Rophynol +100mg/kg of Vitamin C)	199.50±7.50 *	271.50±8.50*	300.00±0.00 *
Group D (1mg/kg of Rophynol +100mg/kg of Vitamin E)	106.50±5.50*	266.50±8.50*	300.00±0.00 *
Group E (1mg/kg of Rophynol +100mg/kg of Vitamin C&E)	128.50±5.50*	244.50±3.50*	265.00±7.00*
F-value	**5.40**	**8.36**	**9.62**

Data was analyzed using ANOVA followed by multiple comparison using Fisher's LSD, and values were considered significant at *p<0.05*. (*= significant, a: not significant).

Table 1: Result revealed a significant decrease in time following wire function test at Day 1 in group B compared to A (*p=0.021*), while groups C, D, and E compared to B (*p=0.020, p=0.011, p=0.012*) showed a significant increase in the motor time at Day 1. Day 2, the motor function test using wire test showed a significant decrease in group B compared to A (*p=0.002*), groups C, D, and E showed a (*p=0.002, p=0.010, p=0.002*) showed a significant increase compared to group B. At day 3, the motor function test using wire test showed a significant decrease in group B compared to A (*p=0.011*), groups C, D, and E showed a (*p=0.012, p=0.013, p=0.014*) showed a significant increase compared to group B.

Effect of vitamins C and E on oxidative stress marker and antioxidants activity following Rohypnol induced brain dysfunction

Table 2: Effect of vitamins C and E on oxidative stress marker and antioxidants activity following Rophynol induced brain dysfunction

	Malondialdehyde level (um/L)	Superoxide Dismutase (U/ml)	Glutathione Peroxidase (Um/ml)	Reduced glutathione (Um/ml)
	MEAN±SEM	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group A (control)	0.71±0.11	23.39±0.38	3.40±0.79	10.17±0.28
Group B (1mg/kg of Rophynol)	0.70±0.20[a]	23.34±0.04[a]	6.05±0.79[a]	10.20±0.06[a]
Group C (1mg/kg of Rophynol +100mg/kg of Vitamin C)	0.69±0.01[a]	20.87±0.81[a]	6.06±0.00[a]	10.12±0.22[a]
Group D (1mg/kg of Rophynol +100mg/kg of Vitamin E)	0.58±0.01[a]	11.14±0.93[a]	8.15±0.26[a]	10.17±0.08[a]
Group E (1mg/kg of Rophynol +100mg/kg of Vitamin C&E)	0.74±0.00[a]	9.01±0.04[a]	18.68±2.89*	10.34±0.11[a]
F-value	**0.34**	**143.04**	**17.61**	**0.24**

Data was analyzed using paired T-test, and values were considered significant at *p<0.05*. BWC: Body weight changes. (*= significant, a: not significant)

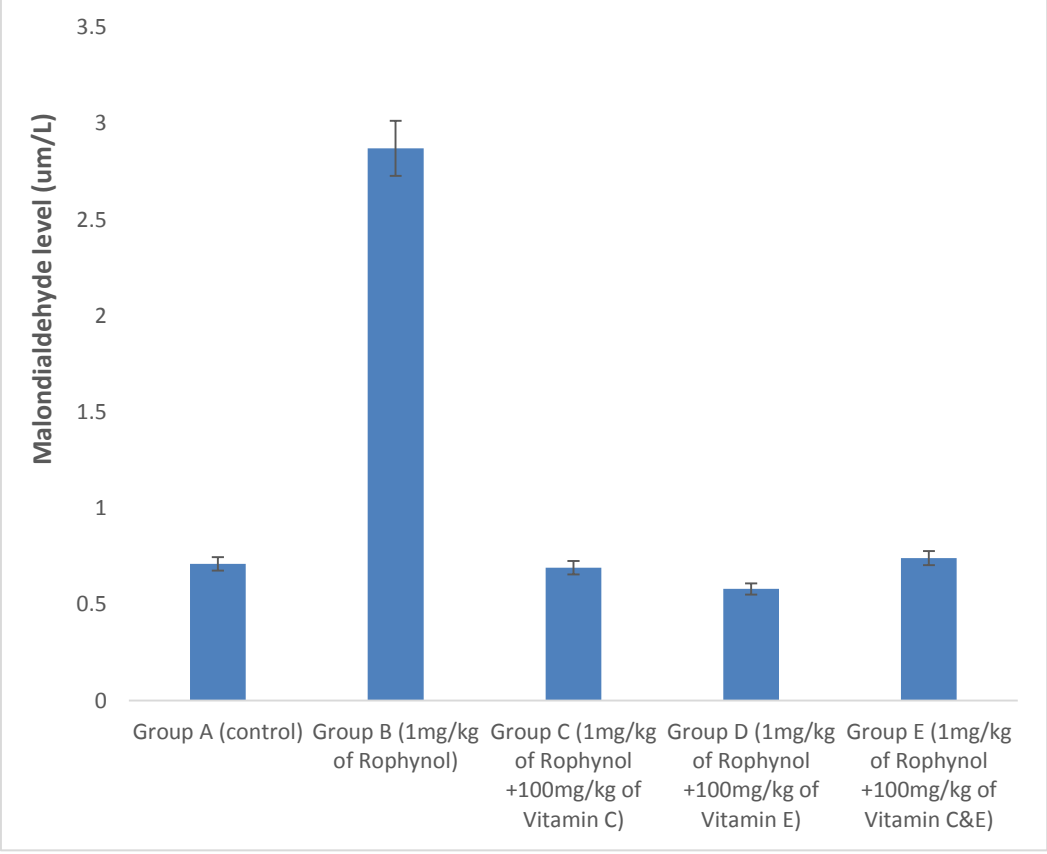

Fig 1: Effect of vitamins C and E on MDA level

Fig 1: Result showed a significant increase in the MDA level in group A compared to B (*p=0.9021*), while groups C, D and E (*p=0.011, p=0.010, p=0.002*) had a significant decrease compared to group B.

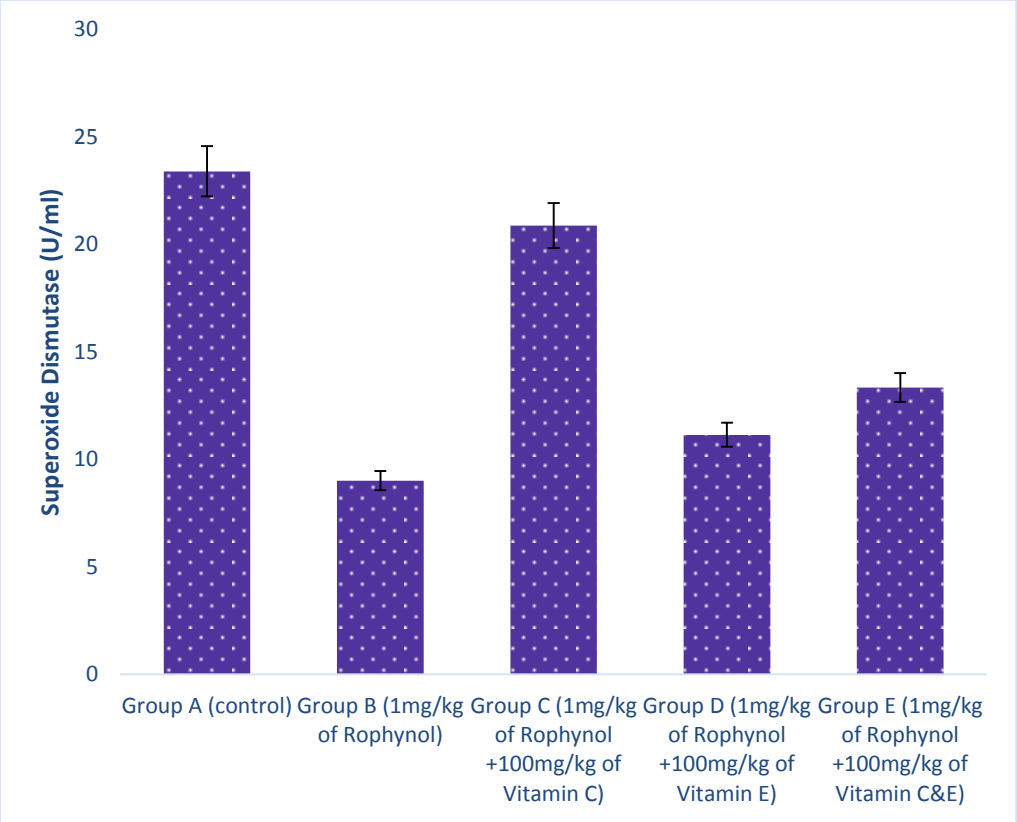

Fig 2: Effect of vitamins C and E on SOD levels

Fig 2: The SOD level result showed a significant decrease in group B compared to A (*p=0.09*), groups C, D, and E (*p=0.028, p=0.00, p= 0.00)* had a significant increase compared to group B.

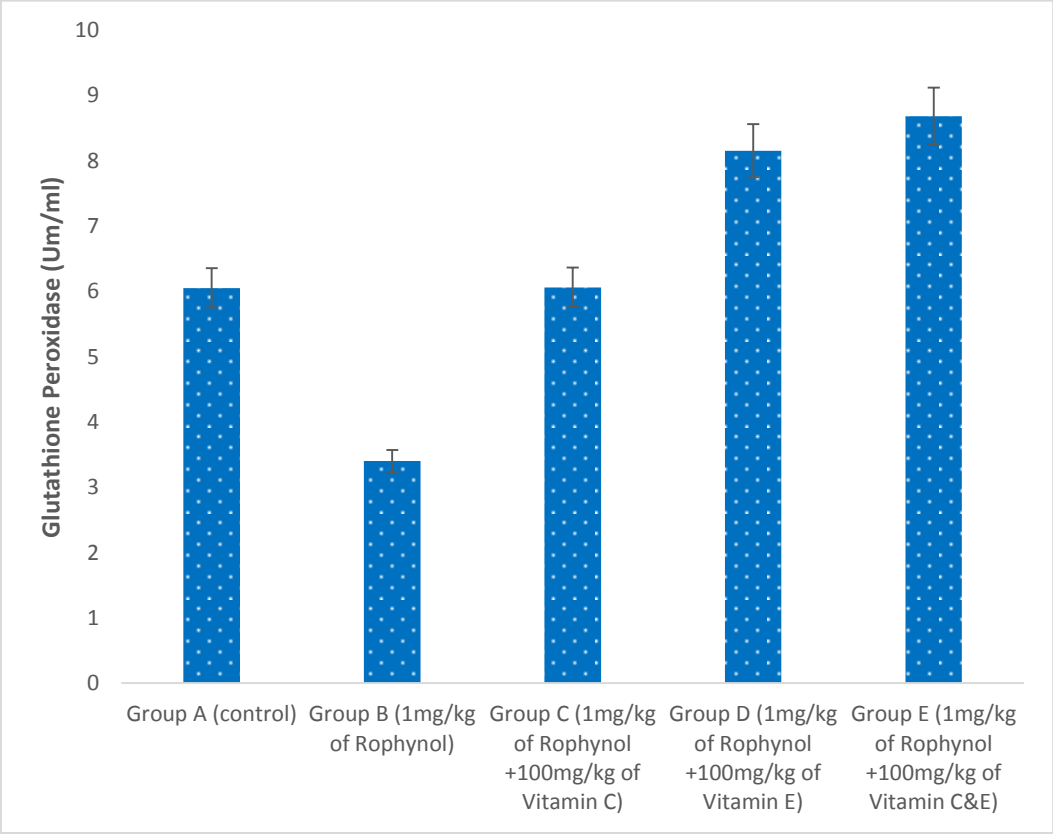

Fig 3: Effect of vitamins C and E on GPx level

Fig 3: The GPx level result showed a significant decrease in group A compared to B (*p=0.024*), while groups C, D and E (*p=0.003, p=0.033, p=0.000*) had a significant increase compared to group B.

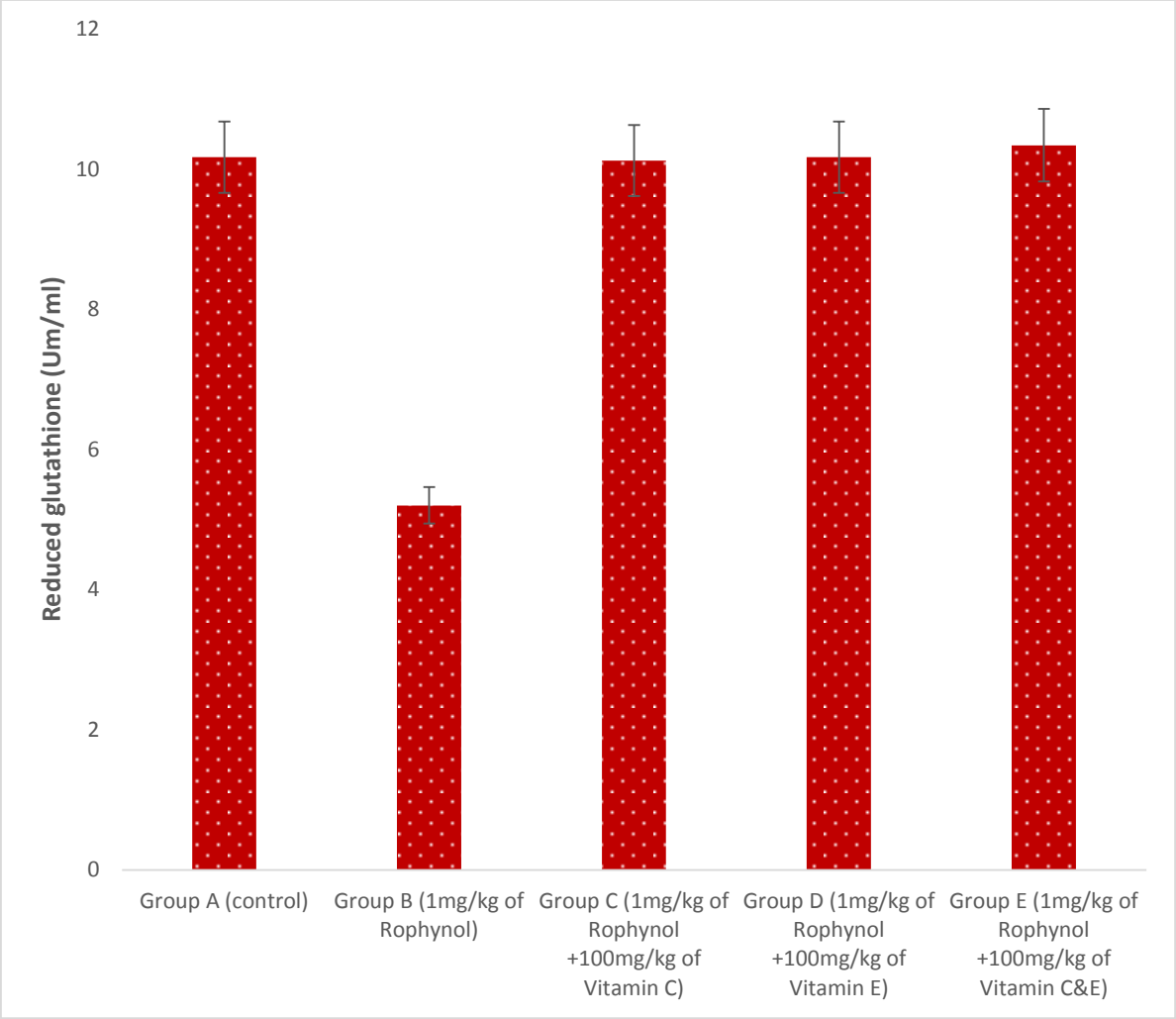

Fig 4: Effect of vitamins C and E on GSH level

Fig 4: The GSH result showed a significant decrease in group A compared to B (*p=0.010*), while groups C, D and E (*p=0.83, p=1.00*) had a significant increase compared to group B.

4.3 HISTOLOGICAL FINDINGS

Substantia Nigra Hematoxyline and Eosine (H&E) Expression

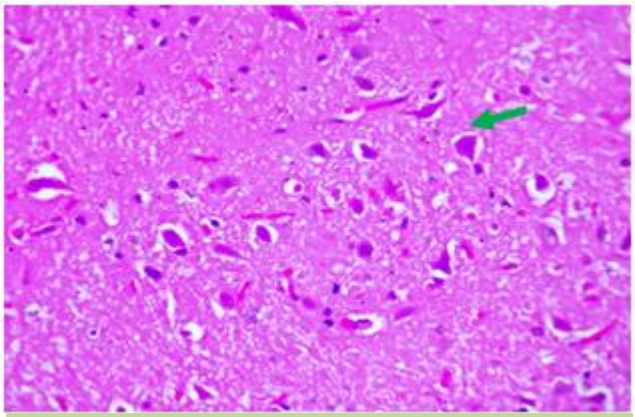

Plate 1 (Group A)- A photomicrograph section of Substantia Nigra showing normal Neuronal cells (↑). Stained by H & E (X400)

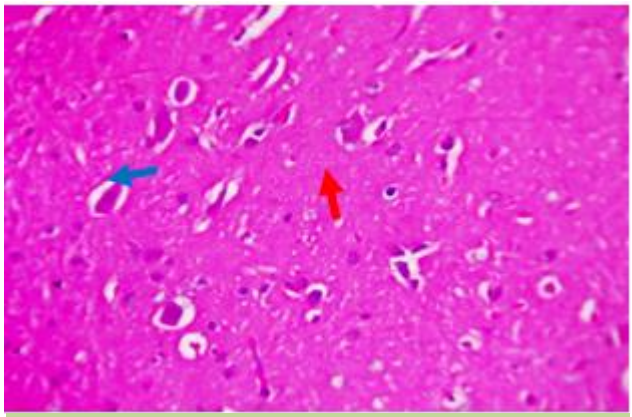

Plate 2 (Group B)- Photomicrograph section of Substantia Nigra showing normal Neuronal cells (↑) and onset of ground glass opacity (↑). Stained by H & E (X400)

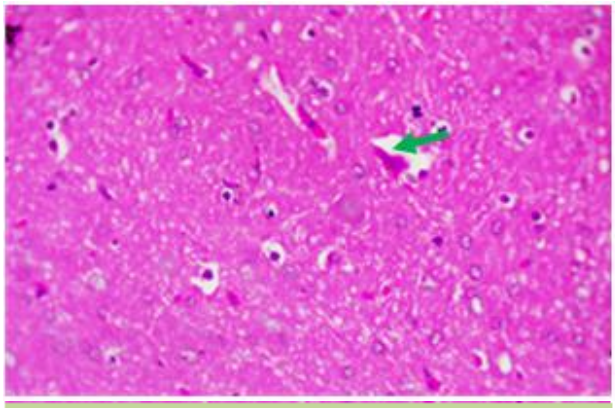

Plate 3 (Group C)- A photomicrograph section of Substantia Nigra showing normal Neuronal cells ( ). Stained by H & E (X400)

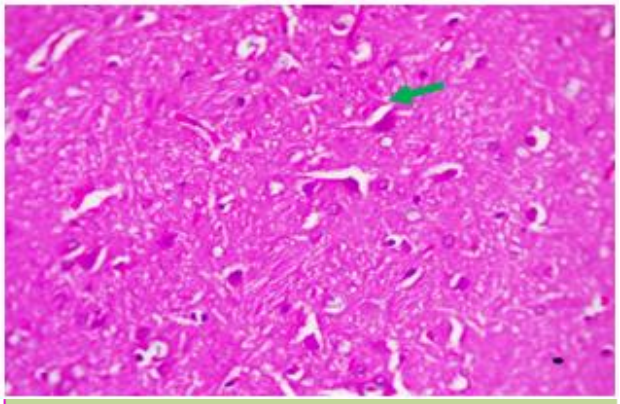

Plate 4 (Group D)- A photomicrograph section of Substantia Nigra showing normal Neuronal cells ( ). Stained by H & E (X400)

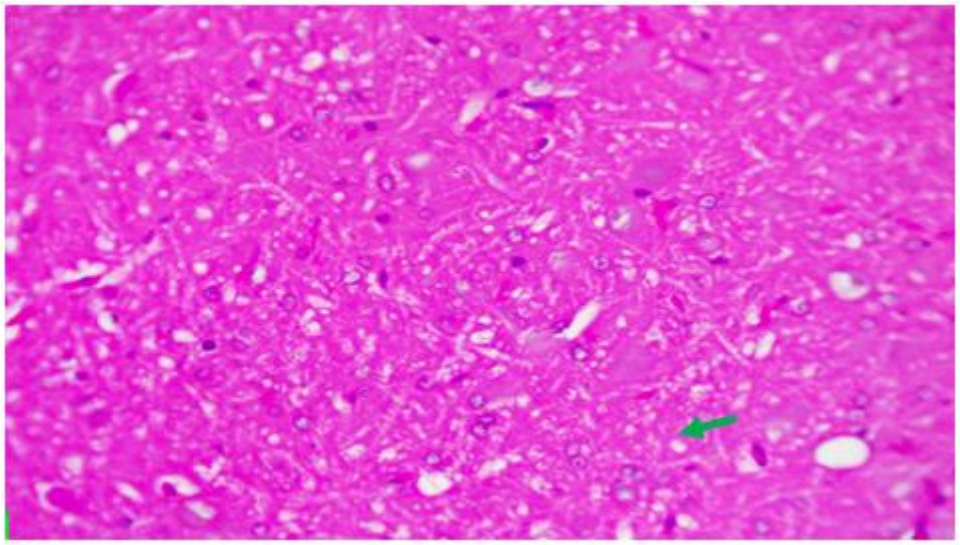

Plate 5 (Group E)- A photomicrograph section of Substantia Nigra showing normal Neuronal cells ( ). Stained by H & E (X400)

The plate 1-5, above presents the tissue architecture of substantia nigra in the five groups of animal. All the experimental groups showed normal neuronal cells while the group B showed the onset of ground glass opacity, which is a negative extracellular deviation from group A. The other experimental groups showed a reversal from opacification.

Substantia Nigra Bielschowsky Silver (BS) Expression

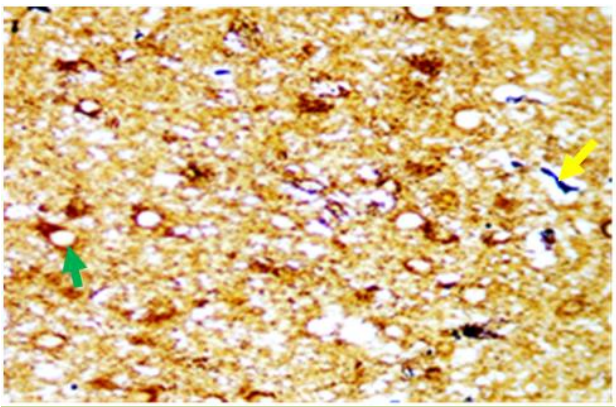

Plate 6 (Group A)- A photomicrograph section of Substantia nigra showing normal dendrites ( ) and axonal fibers ( ). Stained by B & S (X400)

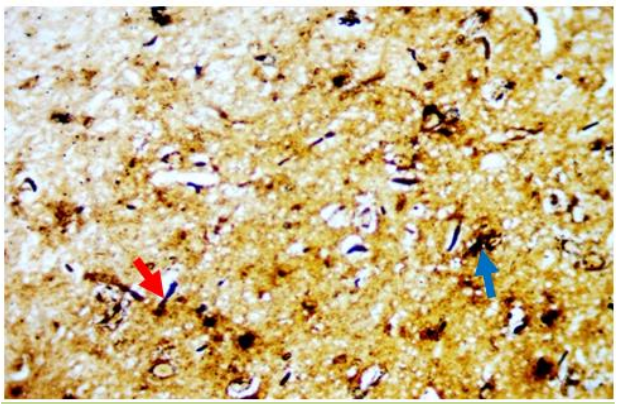

Plate 7 (Group B)- A photomicrograph section of Substantia nigra showing neurofibrillary tangle ( ) and senile plaque ( ). Stained by B & S (X400)

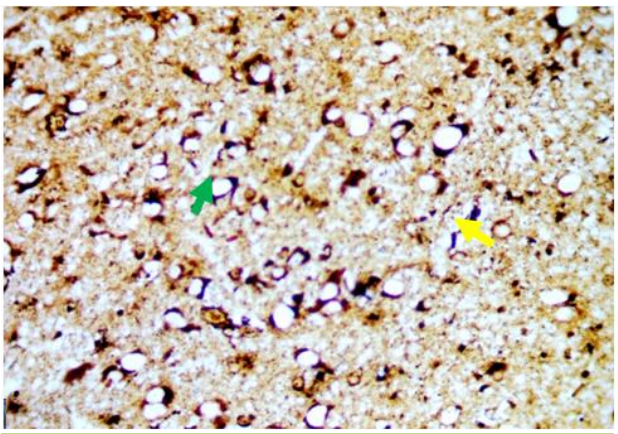

Plate 8 (Group C)- A photomicrograph section of Substantia nigra showing normal dendrites (↑) and axonal fibers (↑). Stained by B & S (X400)

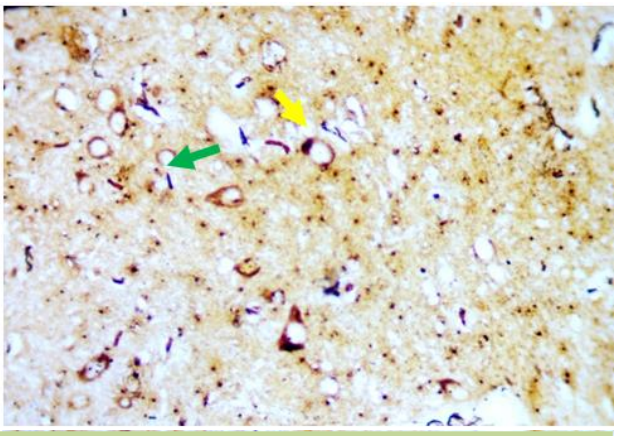

Plate 9(Group D)- A photomicrograph section of Substantia nigra showing normal dendrites (↑) and axonal fibers (↑). Stained by B & S (X400)

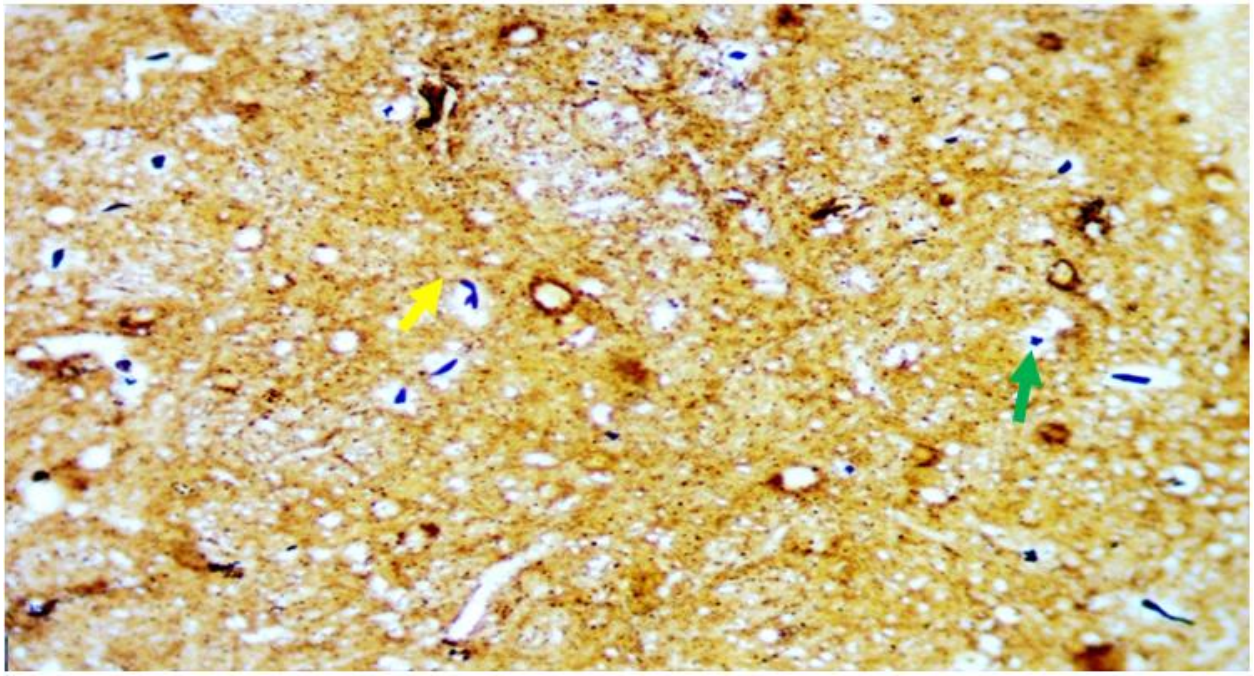

Plate 10 (Group E)- A photomicrograph section of Substantia nigra showing normal dendrites (↑) and axonal fibers (↑). Stained by B & S (X400)

The photomicrograph plate 6-10 above, presents the expression of Bielschowsky Silver stain on the substantia nigra of five animal groups. While the group B present a negative deviation from group A, which were marked by the presence of neurofibrillary tangle and senile plagues, groups C, D and E shows a positive reversal, which were marked by the presence of normal dendrites and axonal fibers.

Discussion

Glutathione, glutathione peroxidase (an enzyme), superoxide dismutase and malondialdehyde were the oxidative stress biomarkers used in this study.

Based on this biochemical assessment study carried out, it was deduced that a significant decrease in the stress oxidative biomarker, glutathione levels when comparing the control group A and group induced with rophynol group B only. This findings agreed to the studies demonstrated by Sechi *et al.* (1996), who administered glutathione intravenously (600 mg twice daily for 30 days) to nine individuals with Parkinson's disease and it was reported significantly improved but in disagreement to the study performed by Djukic-cosic *et al.* (2007) who reported an increase of DNA damage in presence of antioxidants as glutathione and vitamin C in in-vitro studies. There was a decrease in the glutathione level of the only rophynol induced rats substantia nigra as well as rophynol induced group (group B) while there showed an increase in the glutathione marker level when comparing the rophynol induced group B with vitamin treated groups of C, D and E. This is in agreement to Zervos *et al.* (2011) who stated that vitamins C and E can act in a preventive way and moderate the effect of OP (endosulfan) toxicity on lipid peroxidation in the adult rat brain.

Glutathione peroxidase (GP_X) from the result above showed an incessant increase in serum across groups (C, D, and E). The activities of examined enzymes significantly up-regulated to the highest value of glutathione peroxidase in group C, D and vitamins C and E supplemented (group E) when compared to rohypnol induced group B and control group A. Such elevation aligned with the study of Adikwu and Deo, (2013), which explain the antioxidant role of vitamin C and E to minimize the lipid peroxidation and production of ROS. It was concluded that from the research executed by Ifayanti, (2019) also that vitamin C and E can increase the glutathione peroxidase activity in rats exposed to carbon tetrachloride and likewise with this present study of rohypnol toxicity study to substantia nigra and also a decrease in glutathione peroxidase levels when comparing group A (control group) and rohypnol induced group B which also went in alignment to the study of Ifayanti, (2019).

Superoxide dismutase (SOD) protects the cell from the oxidative damage by catalyzing the superoxide into the oxygen and hydrogen peroxide. From this study, it was notable that there were significant increase in the serum level of Superoxide dismutase (SOD) when comparing group C, D and E with group B (the rophynol induced substantia nigra group) which went in agreement to the postulated study of Abdel-Hafez *et al.* (2010) which states that an increase in the SOD activity may reduce the lipid peroxidation and estimation of its activity and its relation with the lipid peroxidation. There was a significant decrease in the level of Superoxide dismutase when comparing the control group A and the rophynol induced group B which indicated that they was an increase in oxidative stress which led to a corresponding increase in lipid peroxidation. This finding coincided with the study of Rao *et al.* (2021) that explained that a decrease in superoxide dismutase level indicates an increased level of lipid peroxidation.

Malondialdehyde (MDA) is one of the most important stress biomarker because it is one of the end-products of lipid peroxidation. From this study, it was observed that there was a significant increase in the MDA serum levels when comparing group A (rohynol induced) to group B. As well, this concurrently showed that there was an inducing of oxidative stress which decreased the malondialdehyde level, thus, elevated lipid peroxidation which will affect the integrity of cell membranes. This result statement went in concordance to the study carried out by Irene et al. (1992) which explain how reactive oxygen specie (ROS) when produced in much amount and are accumulated can cause damage and alter the cell membrane. Also, when considering the group B (rophynol induced) and other groups treated with supplements, group C, D and E, there happened to be a significant decrease in the Malondialdehyde serum levels in which this findings went in support with the research carried out by Abdollahzad *et al.* (2009) which stated that every day supplementation with 250 mg vitamin C for 12 weeks increases serum vitamin C, decreases MDA levels, and improves lipid profiles in hemodialysis patients. From the result above, it can be worthy of note that there was the most effective treatment of rophynol in group treated with vitamin E only (group D), which was parallel the study carried out by Wang *et al.* (2000) which stated that vitamin E partitions into lipoproteins and cell membranes, where it represents a minor constituent of most membranes. It has a major function in its action as a lipid antioxidant to protect the polyunsaturated membrane lipids against free radical attack. .

These studies above all tallied to the histological findings of this research, thus promoting the antioxidant ameliorative effects of the vitamin supplemented rophynol induced substantia nigra when compared to the only induced rohypnol group B.

Conclusion

This study have shown that good antioxidants such as vitamin C and E are very much effective in reducing oxidative stress and thus, have great antioxidant ameliorative properties to the Rophynol induced substantia nigra of the wistar rats

Recommendation

From this finding, it can be deduced that vitamin C and E have good antioxidant attributes and are good sources of treatments that can be given or administration to patients by health professionals that abused the drug, flutrinazepam (rophynol) in the toxic levels. This should be done in other to prevent and preserve surrounding cell membrane of dopaminergic neurons and the substantia nigra as a whole against peroxidation which can lead to randicity and progressive degeneration of cells of the substantia nigra.

References

1. Abdel-Hafez, H. Z., Mohamed, E. E., & Abd-Elghany, A. A. (2010). Tissue and blood superoxide dismutase activity and malondialdehyde level in leprosy. *Journal of the European Academy of Dermatology and Venereology*, *24*(6), 704-708.
2. Abdollahzad, H., Eghtesadi, S., Nourmohammadi, I., Khadem-Ansari, M., Nejad-Gashti, H., & Esmaillzadeh, A. (2009). Effect of vitamin C supplementation on oxidative stress and lipid profiles in hemodialysis patients. *International journal for vitamin and nutrition research*, *79*(56), 281-287.
3. Adikwu, E., & Deo, O. (2013). Hepatoprotective effect of vitamin C (ascorbic acid).
4. Djukic-Cosic, D., Bulat, Z. P., Ninkovic, M., Malicevic, Z., & Matovic, V. (2007). Effect of subacute cadmium intoxication on iron and lipid peroxidation in mouse liver. *Toxicology Letters*, (172), S209.
5. Dzirasa, K., Ribeiro, S., Costa, R., Santos, L. M., Lin, S. C., Grosmark, A., ... & Nicolelis, M. A. (2006). Dopaminergic control of sleep–wake states. *Journal of Neuroscience*, *26*(41), 10577-10589. doi:10.1523/JNEUROSCI.1767-06.2006. PMC 6674686. PMID 17035544
6. Ifayanti, T. (2019). Effect of Vitamin C & E on Enzyme Glutathione Peroxidase Activity (GSH-Px) in Male Wistar Strain Rats Exposed to Carbon Tetrachloride (CCl4). *International Journal of Advancement in Life Sciences Research*, 12-20.
7. Ceballos-Picot, I., Trivier, J. M., Nicole, A., Sinet, P. M., & Thevenin, M. (1992). Age-correlated modifications of copper-zinc superoxide dismutase and glutathione-related enzyme activities in human erythrocytes. *Clinical Chemistry*, *38*(1), 66-70.

8. Rao, Y. L., Ganaraja, B., Marathe, A., Manjrekar, P. A., Joy, T., Ullal, S., ... & Murlimanju, B. V. (2021). Comparison of malondialdehyde levels and superoxide dismutase activity in resveratrol and resveratrol/donepezil combination treatment groups in Alzheimer's disease induced rat model. *3 Biotech*, *11*, 1-10.
9. Sechi, G., Deledda, M. G., Bua, G., Satta, W. M., Deiana, G. A., Pes, G. M., & Rosati, G. (1996). Reduced intravenous glutathione in the treatment of early Parkinson's disease. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *20*(7), 1159-1170.
10. Wang, X., & Quinn, P. J. (1999). Vitamin E and its function in membranes. *Progress in lipid research*, *38*(4), 309-336.
11. Zervos, I. A., Nikolaidis, E., Lavrentiadou, S. N., Tsantarliotou, M. P., Eleftheriadou, E. K., Papapanagiotou, E. P., ... & Taitzoglou, I. A. (2011). Endosulfan-induced lipid peroxidation in rat brain and its effect on t-PA and PAI-1: ameliorating effect of vitamins C and E. *The Journal of toxicological sciences*, *36*(4), 423-433.
12. da Cunha Germano, B. C., de Morais, L. C. C., Idalina Neta, F., Fernandes, A. C. L., Pinheiro, F. I., do Rego, A. C. M., ... & Cobucci, R. N. (2023). Vitamin E and its molecular effects in experimental models of neurodegenerative diseases. *International Journal of Molecular Sciences*, *24*(13), 11191. https:// doi.org/10.3390/ijms241311191
13. Regner-Nelke, L., Nelke, C., Schroeter, C. B., Dziewas, R., Warnecke, T., Ruck, T., & Meuth, S. G. (2021). Enjoy carefully: the multifaceted role of vitamin E in neuro-nutrition. *International Journal of Molecular Sciences*, *22*(18), 10087. Doi: 10.3390/ijms221810087. PMID: 34576251; PMCID: PMC8466828.vol.22,issue 18
14. Danboyi, T., Abdullazeez, J., Alhassan, A. W., & Hassan-Danboyi, E. (2019). Protective effect of co-administration of vitamins C and E on reserpine-induced oxidative stress in mice. *Journal of African Association of Physiological Sciences*, *7*(1), 30-37.
15. Rang, H. P., Dale, M. M., Ritter, J. M., Flower, R. J., & Henderson, G. (2011). *Rang & Dale's pharmacology*. Elsevier Health Sciences.
16. Katzung, B. G. (2017). *Basic and clinical pharmacology 14th edition*. McGraw Hill Professional.
17. Brunton, L. L., Hilal-Dandan, R., & Knollmann, B. C. (2018). The pharmacological basis of therapeutics. *Goodman*, *11*, 1280-1281.
18. Murray, S. J., Black, B. L., Reid, S. J., Rudiger, S. R., Bawden, C. S., Snell, R. G., ... & Faull, R. L. (2019). Chemical neuroanatomy of the substantia nigra in the ovine brain. *Journal of chemical neuroanatomy*, *97*, 43-56. Doi: 10.1016/j.jchemneu.2019.01.007. Epub 2019 Jan 18. PMID: 30664938.
19. Nutt, D. J., & Blier, P. (2016). Neuroscience-based nomenclature (NbN) for journal of psychopharmacology. *Journal of Psychopharmacology*, *30*(5), 413-415.
20. Rohypnol. DEA Drug Fact Sheet. Accessed June 9, 2020 at https://www.dea.gov/factsheets/rohypnol
21. Crisis, O. O. (2020). National Institute on Drug Abuse. *URL: https://www. drugabuse. gov/drug-topics/opioids/opioid-overdose-crisis [accessed 2021-05-01]*. https://www.drugabuse.gov/sites/default/files/drugfacts_clubdrugs_12_2014.pdf
22. Szaflarski, M., & Sirven, J. I. (2017). Social factors in marijuana use for medical and recreational purposes. *Epilepsy & Behavior*, *70*, 280-287. Doi: 10.1016/j.yebeh.2016.11.011. Epub 2017 Jan 26. PMID: 28132734.
23. Buchanan-Peart, K. A. R., Oribhabor, G. I., Khokale, R. V., Nelson, M. L., & Cancarevic, I. (2020). Cannabis, More Than the Euphoria: Its Therapeutic Use in Drug-Resistant Epilepsy. *Cureus*, *12*(7). Doi: 10.7759/cureus.9299. PMID: 32832296; PMCID: PMC7437102.
24. Baldwin, D. S. (2022). Clinical management of withdrawal from benzodiazepine anxiolytic and hypnotic medications. *Addiction*, *117*(5), 1472-1482. Doi: 10.1111/add.15695. Epub 2021 Oct 28. PMID: 34542216.
25. Bohmwald, K., Andrade, C. A., Gálvez, N. M., Mora, V. P., Muñoz, J. T., & Kalergis, A. M. (2021). The causes and long-term consequences of viral encephalitis. *Frontiers in Cellular Neuroscience*, *15*, 755875. Doi: 10.3389/fncel.2021.755875. PMID: 34916908; PMCID: PMC8668867.
26. Thomas, J. C., & Kopel, J. (2023). Male victims of sexual assault: a review of the literature. *Behavioral Sciences*, *13*(4), 304. Doi: 10.3390/bs13040304. PMID: 37102818; PMCID: PMC10135558.
27. Akagi, M., Masaki, S., Kitazumi, K., Mio, M., & Tasaka, K. (1991). Comparative study of the adverse effects of various radiographic contrast media, including ioversol, a new low-osmolarity medium. II. The complement system and endothelial cells. *Methods and findings in experimental and clinical pharmacology*, *13*(7), 449-454.