

Research Article

## Protective effect of co-administration of vitamins C and E on reserpine-induced oxidative stress in mice

T. Danboyi<sup>1</sup>, J. Abdullazeez, <sup>2</sup>A.W. Alhassan<sup>2</sup> and E. Hassan-Danboyi<sup>2</sup>

Departments of Human Physiology, <sup>1</sup>Kaduna State University, Kaduna, Nigeria and <sup>2</sup>Ahmadu Bello University, Zaria, Nigeria

**Keywords:**

Co-administration, neuroprotective, oxidative, reserpine-induced, stress

**ABSTRACT**

**Background:** Several studies have shown potential benefits of antioxidants in the treatment of Parkinson's disease (PD) but none have combined vitamins C and E targeting the oxidative stress (OS). **Aim:** To evaluate the neuroprotective effect of co-administration of vitamins C + E or single vitamin, on parameters of reserpine-induced OS in mice. **Methods:** Twenty-five mice were randomly assigned into 5 groups.: Group I received only distilled water (control); other groups received reserpine 0.1 mg/kg intraperitoneally on alternate days. In addition, Group III received vitamin E 200 mg/kg/day orally; group IV, had vitamin C 250 mg/kg/day orally and group V, had both vitamins orally. All drugs were given concurrently for 28 days. The mice were humanely sacrificed and brain homogenate made to assess for biomarkers of OS. Data were expressed as mean  $\pm$  SEM and values at  $p < 0.05$  were considered significant. **Results:** The significant increase in malondialdehyde concentrations observed in the Res group ( $42.2 \pm 0.28$  Umol/L) compared to control ( $37.54 \pm 1.27$  Umol/L), was ameliorated in all the vitamin-treated groups with significance in the Res+Vit C group ( $35.0 \pm 1.69$  Umol/L) compared to the Res group ( $p=0.002$ ). Superoxide dismutase (SOD) activity increased significantly ( $p=0.003$ ) across the vitamin-treated groups ( $24.9 \pm 2.11$  Umol/mg,  $24.0 \pm 1.78$  Umol/mg and  $22.4 \pm 1.50$  Umol/mg in the Res+Vit E, Res+Vit C and co-administered groups respectively) compared to control ( $14.3 \pm 1.65$  Umol/mg), with non-significant increase in the Res group ( $20.6 \pm 1.42$  Umol/mg); catalase activity increased significantly in the Res+Vit C ( $28.0 \pm 3.70$  Umol/mg) and co-administered ( $30.2 \pm 2.22$  Umol/mg) groups compared to controls ( $14.3 \pm 1.65$  Umol/mg) and Res ( $20.6 \pm 1.42$  Umol/mg) groups ( $p=0.000$ ), with non-significant increase in the Res+Vit E group ( $17.6 \pm 0.68$  Umol/mg). The highest GSH level was seen in the Res group ( $45.2 \pm 2.65$  Umol/mgpr) and the lowest level seen in the Res+Vit E group ( $38.58 \pm 1.78$  Umol/mgpr) with no significant difference across all the groups ( $p=0.104$ ). **Conclusion:** The co-administration of vitamins C and E fails to confer significant superior neuroprotection against reserpine-induced OS compared to single vitamin administration.

© Copyright 2019 African Association of Physiological Sciences -ISSN: 2315-9987. All rights reserved

### INTRODUCTION

Parkinson's disease (PD), first described by James Parkinson (1817), is the most common movement disorder affecting about 1.5% of the world's population over 65 years of age (~10million) (Blesa and Przedborski, 2014). This prevalence increases with advancing age to about 5% in those above 85 years (Farn, 2003) and the prevalence is projected to double by 2040 due to increasing life expectancy with higher

aging population and increasing industrialization (Dorsey *et al.*, 2018). It is the second most common neurodegenerative disease (NDD) (Tanner and Aston, 2000; Lee *et al.* 2009). Neurological disorders are now noted to be the leading cause of disability worldwide, among which, PD is the fastest growing (Dorsey and Bloem, 2018).

Several conventional (Schapira, 2005; Clark, 2007; Davie, 2008; Weinreb *et al.*, 2010; Thomas, 2017) and other treatment strategies such as nicotine (Barreto *et al.*, 2015), non-steroidal anti-inflammatory drugs, NSAIDs (Wahner *et al.*, 2007; Gagne *et al.*, 2010), docosahexanoic acid (Ozsoy *et al.*, 2011), caffeine

\*Address for correspondence:

Email [timothy.danboyi@kasu.edu.ng](mailto:timothy.danboyi@kasu.edu.ng)

(Joghataie *et al.*, 2004; Saaksjarvi *et al.*, 2008) etc. have been explored in the treatment of PD but some of these findings for example nicotine, are controversial (Ferrea and Winterer, 2009). Even the gold standard in PD treatment, l-dopa and carbidopa (a peripheral decarboxylase inhibitor), is associated with dyskinesia and end-of-dose “wearing-off” (Fahn, 1999) and it has been suggested that l-dopa might be neurotoxic, hastening the progression of PD via oxidative stress (Cracium *et al.*, 2016). Although the motor symptoms initially respond well to pharmacologic therapies, none has been shown to slow down or halt the disease progression (Elmer and Hauser, 2013). Hence, the need to explore newer neuroprotective agents that can target the main pathophysiology of the disease with the aim of preventing further damage or neuronal loss, halting the progression, ameliorating symptoms and initiating repair or healing processes.

Given the well-established central role of oxidative stress (OS) in the pathogenesis of PD (Jenner, 2003), the focus in PD therapy is now shifting to antioxidants such as vitamins E and C, which might protect against oxidative damage by neutralising free radicals. Both vitamins C and E had been shown to have neuroprotective effects as individual antioxidants in some studies (Roghani and Behzadi, 2001; Miyake *et al.*, 2011; Harrison, 2012) but the co-administration of both vitamins was shown to be of greater efficacy against oxidative stress and nephrotoxicity in rodents (Khadkhodae *et al.*, 2008), with more potent antidiabetic effects (Tanko *et al.*, 2014), although, in another study, this was shown to be otherwise (Mohammed *et al.*, 2015). The combined administration of the two vitamins has been shown to potentiate the effect of each other on reserpine-induced oral dyskinesia in rodents (Faria *et al.*, 2005), also suggesting that the combination of both vitamins provide a complete antioxidant defence (Mahadik *et al.*, 2001).

Vitamin C plays an important role in recycling of vitamin E (Kohen and Nyska, 2002; Harrison, 2012), a process that results in the formation of vitamin C (semi-ascorbyl) radical (Powers and Jackson, 2008; Pillai and Yao, 2015). Therefore, a combination of the two vitamins, due to their synergistic interaction (Baptista-Ortega and Ruiz-Feria, 2010; Yarube and Ayo, 2011; Dawud *et al.*, 2014; Bursac-Metrovic *et al.*, 2016), may enhance their antioxidant benefit. The aim of this study is to evaluate the effects of co-administration of vitamin C (*l*-ascorbic acid) and vitamin E ( $\alpha$ -tocopherol) on reserpine-induced oxidative stress and parkinsonism in mice.

## METHODS

Vitamins C and E and reserpine were of analytical grade and purchased from MedChem Express, U.S.A. The reserpine was dissolved in 0.5% glacial acetic acid, vitamin E ( $\alpha$ -tocopherol acetate) in dimethyl sulfoxide and vitamin C (*l*-ascorbic acid) in distilled water.

### *Ethical Consideration*

Ethical clearance/approval was sought from the Ahmadu Bello University Ethical Committee on Research Animals Use and Care. The study was conducted in accordance with the guidelines of the A.B.U Animals Use and Care Policy.

### *Experimental Animals*

A total of 25 mice, 8-12 weeks old and weighing 20-30g were housed in plastic cages under standard environmental conditions with free access to commercial grower mash feed and water.

### *Induction of Parkinsonism*

Parkinsonism was induced by alternate day's administration of 0.1mg/kg reserpine injection *i.p* over 4weeks (Sarmiento-Silva *et al.*, 2014).

### *Animal Grouping*

The mice were randomly assigned into five (5) groups of 5 animals each:

Group I (n=5): were fed with normal diet *ad libitum* plus normal saline (1ml/kg) throughout the study period. This group served as normal control.

Group II (n=5): received reserpine (0.1mg/kg) *i.p* on alternate days (Sarmiento-Silva *et al.*, 2014) for 4 weeks. They served as positive control.

Group III (n=5): were pre-treated with vitamin C (250mg/kg) *i.p* daily plus reserpine (0.1mg/kg) *i.p* on alternate days for 4 weeks.

Group IV (n=5): were pre-treated with vitamin E (200mg/kg) *i.p* daily plus reserpine (0.1mg/kg) *i.p* on alternate days for 4 weeks.

Group V (n=5): were pre-treated with vitamin C (250mg/kg) *i.p* and vitamin E (200mg/kg) *i.p* daily plus reserpine (0.1mg/kg) *i.p* on alternate days for 4weeks.

### *Homogenate Preparation*

The mice were humanely sacrificed by anaesthetizing them with *i.p* ketamine (10 mg/kg) and *i.p* diazepam (2 mg/kg). As soon as the effect of anaesthesia is evident, the brain was carefully harvested and a homogenate was prepared as described by Freitas *et al.* (2005), using phosphate buffer solution. The homogenate was

used for analysis of biomarkers for OS (according to the manufacturer's manual/guidelines).

*Assay for Biomarkers of Oxidative Stress*

*Protocol for assay of superoxide dismutase (SOD) activity*

Activity of SOD was carried out according to the method described by Misra and Fridovich (1972). The assay is based on the principle of superoxide dismutase (SOD) inhibition of auto-oxidation of adrenaline at pH of 10.2. 0.2 ml of diluted homogenate (0.1ml of homogenate plus 0.9ml of distilled water i.e. dilution factor of 1:10) was mixed with 2.5 ml of 0.05 mM carbonate buffer (14.3 g of Na<sub>2</sub>CO<sub>3</sub> and 4.2 g NAHCO<sub>3</sub> plus 1,000 ml distilled water at pH of 10.2) and 0.3 ml of 0.3mM adrenaline (0.01 g of adrenaline plus 17 ml of distilled water). The absorbance was measured over 60s at 450 nm. The % inhibition was calculated as:

$$\% \text{ Inhibition of adrenaline oxidation} = \frac{\text{Increase in substrate absorbance/min}}{\text{Increase in absorbance of blank/min}} \times 100$$

Hence, the concentration of the SOD activity (in Umol/mg) is the amount of SOD required to elicit 50% inhibition of adrenaline auto-oxidation per minute.

*Protocol for assay of reduced glutathione (GSH) activity*

Reduced glutathione (GSH) activity was carried out according to the method described by Ellman (1959) as modified by Rajagopalan *et al.* (2004). It is based on the reaction of 5,5'-dithiobis nitro benzoic acid (DNTB) with GSH. To 150 uL of the homogenate 1.5 ml of 10% trichloroacetic acid (10g of TCA dissolved in distilled water up to 100 ml in a volumetric flask) was added and centrifuged at 1500 g for 5 minutes. 1ml of the supernatant was mixed with 0.5 ml of Ellman's reagent (19.8g of DNTB in 100ml of 0.1% sodium).

*Protocol for assay of catalase (CAT) activity*

Catalase activity was measured according to the method described by Aebi (1984). The principle is based on the decrease in absorbance of the sample induced by the catalytic decomposition of H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub> in the presence of catalase. This enzymatic decomposition of H<sub>2</sub>O<sub>2</sub> is a first order reaction, the rate of which is directly proportional to the amount of H<sub>2</sub>O<sub>2</sub> present in the homogenate. 2.8 ml of the homogenate was added to 0.1 ml of 30mM H<sub>2</sub>O<sub>2</sub> in a test tube and the rate of decomposition was measured at 240 nm for 5minutes using the spectrophotometer. Catalase activity was calculated using the molar extinction coefficient (E) of 0.041mM<sup>-1</sup>cm<sup>-1</sup> as:

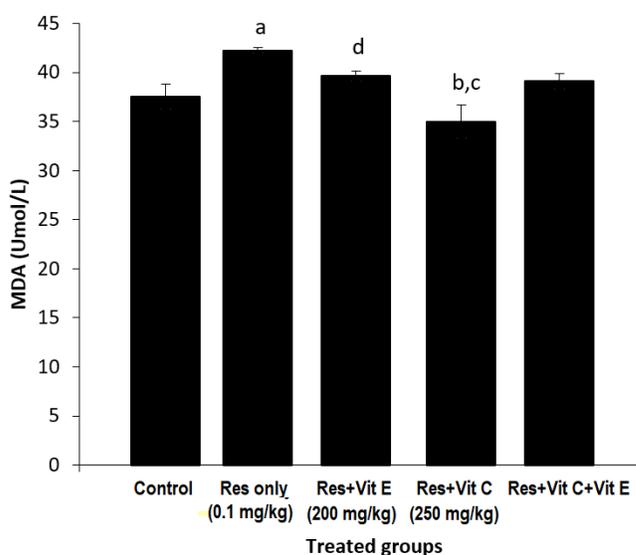
$$\text{Catalase concentration} = \text{absorbance} / E$$

*Protocol for determination of MDA concentrations*

The level of thiobarbituric-acid (TBA) reactive substance (TBARS), as an index of lipid peroxidation was determined by quantitative measurement of MDA concentration based on the method described by Okhawa *et al.* (1979), as modified by Atawodi *et al.* (2011). The principle is based on the reaction of 14% trichloroacetic acid (TCA) and 0.67% TBA solution both of which were added to about 100 µL of the supernatant, forming a TBARS, an adduct that absorbs strongly at 532 nm. The supernatant was deproteinized by addition of the TCA and the mixture was heated in a water bath at 80°C for 30minutes before cooling on ice then centrifuged at 2000 g for 10 minutes. The product of lipid peroxidation, MDA is expected to be released by the deproteinization and react with the TBA forming the coloured product, MDA-TBA (or TBARS), absorbance of which was measured at 532 nm with a UV spectrophotometer. The concentrations of TBARS were calculated as the absorbance/molar extinction coefficient of malondialdehyde: 1.56×10<sup>5</sup> mol/L/cm. All TBARS concentrations are expressed in µmol/g tissue protein.

*Statistical Analysis*

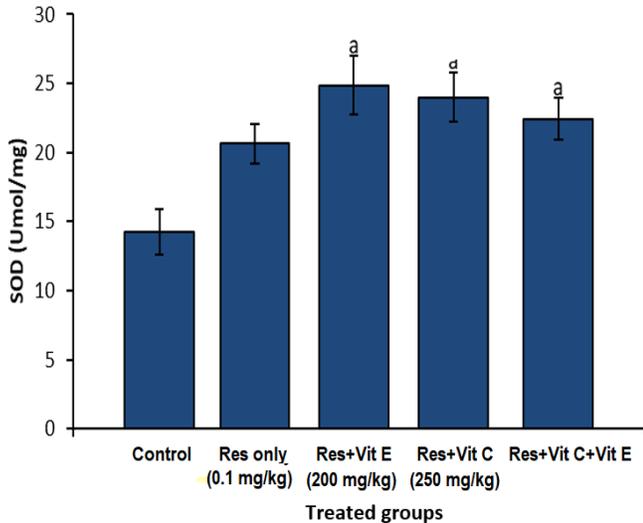
Data were expressed as mean ± SEM and analysed using one-way analysis of variance (ANOVA), followed *Tukey's* post-hoc test. Values at *p*<0.05 were considered statistically significant using SPSS version 25.0.



**Fig. 1.** MDA concentration by group: Res= Reserpine, Vit= vitamin. <sup>a</sup>*p* < 0.05 indicates statistically significant difference compared to control, <sup>b</sup>*p* < 0.05 compared to Res group, <sup>c</sup>*p* < 0.05 compared to Res + Vit E, and <sup>d</sup>*p* < 0.05 compared to Res + Vit C group (One-way ANOVA followed by *Tukey's* post-hoc test).

*Level of SOD activity*

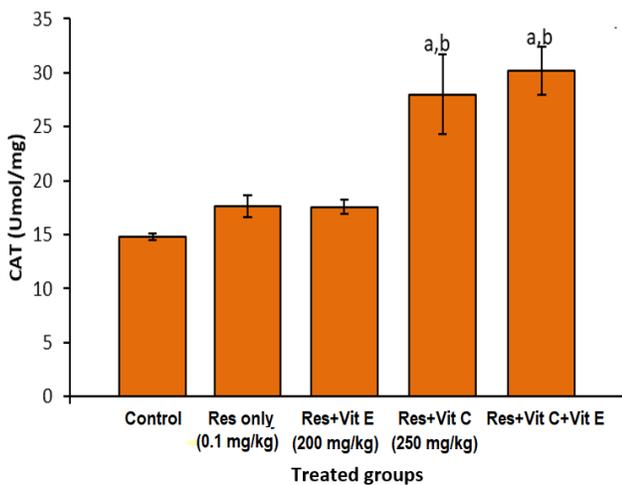
There was a significant increase in the level of SOD activity across the vitamin-treated groups ( $24.0 \pm 1.78$  Umol/mg by vitamin C,  $24.9 \pm 2.11$  Umol/mg by vitamin E and  $22.4 \pm 1.5$  Umol/mg by both vitamins).  $F = 5.660, p = 0.003$ .



**Fig. 2.** Level of SOD activity by group: Res= Reserpine, Vit= vitamin. <sup>a</sup> $p < 0.05$  indicates statistically significant difference compared to the control (One-way ANOVA followed by Tukey's *post-hoc* test).

*Level of CAT activity*

There was significant increase in catalase activity in the Res+Vit C ( $28.0 \pm 3.70$  Umol/mg) and co-administered ( $30.2 \pm 2.22$  Umol/mg) groups compared to both control ( $14.3 \pm 1.65$  Umol/mg) and Res ( $20.6 \pm 1.42$  Umol/mg) groups, with non-significant increase in the Res+Vit E group ( $17.6 \pm 0.68$  Umol/mg).  $F = 2.214, p = 0.000$ .



**Fig. 3.** Level of CAT activity by group: Res = Reserpine, Vit = vitamin. Superscripts <sup>a</sup> $p < 0.05$  indicates statistically significant difference compared to control and <sup>b</sup> $p < 0.05$  compared to Res group (One-way ANOVA followed by Tukey's *post-hoc* test).

GSH level

There was no significant difference in the GSH level across the groups, the highest level seen in the Res group ( $45.18 \pm 2.65$  Umol/mgpr) and the lowest level seen in the Res + Vit E group ( $38.58 \pm 1.78$  Umol/mgpr).  $F = 2.214, p = 0.104$ .

**Table 1:** Reduced glutathione levels by groups

Groups	Reduced Glutathione (GSH) (Umol/mgpr)
Control	39.72 ± 1.45
Res only (0.1 mg/kg)	45.18 ± 2.65
Res + Vit E (200 mg/kg)	38.58 ± 1.78
Res + Vit C (250 mg/kg)	43.08 ± 1.67
Res + Vit C + Vit E	41.82 ± 0.74

Res= Reserpine, Vit= vitamin. No statistically significant difference (One-way ANOVA followed by Tukey's *post-hoc* test).

**DISCUSSION**

Oxidative stress (OS) can be defined as an imbalance between the levels of ROS produced and the ability of the biological system to neutralize them, creating a state of possible cellular damage (Dias *et al.*, 2013). It can also be defined as the presence of ROS in excess of the available antioxidant buffering capacity (Czerska *et al.*, 2015). These ROS may damage DNA, proteins, lipids, proteins and carbohydrates changing the organism's structure and functions (Czerska *et al.*, 2015). There is normally a balance between production of ROS and the antioxidant defence against them and any disturbance of such balance is capable of causing OS. Within the cell where oxygen metabolism takes place, ROS are normally and constantly formed as part of normal physiological processes with the majority of endogenous ROS generated by the mETC during the production of ATP (Eckert *et al.*, 2003). The antioxidant defence consist of enzymatic and non-enzymatic antioxidants.

The mechanism involved in toxin-induced PD in well-established animal models such as MPTP, 6-OHDA, rotenone and paraquat is associated with OS (Dias *et al.*, 2013). Although, apart from PD, OS had also been linked to other NDD such as Alzheimer's disease (AD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) despite having distinct pathological and clinical features (Lin and Beal, 2006), supporting the role of oxidative stress in neurodegeneration (Anderson, 2004).

Markers of lipid peroxidation such as HNE and MDA are increased in the SN of PD patients, while PUFAs

are decreased (Montine *et al.*, 2004). Our result showed a significant evidence of lipid peroxidation (figure 1), characterized by a significant increase in MDA concentration in the reserpine only treated group compared to control. This was however, significantly ameliorated by vitamins C and E as single administrations. This is in line with the findings of Nayak *et al.*, (2018), which showed neuroprotective effect of vitamin E against lipid peroxidation at different regions of rat brain. Surprisingly, vitamin C conferred the greater significant protection against lipid peroxidation, not being a membrane lipid-soluble antioxidant itself. This finding on the co-administered group is unexpected, as vitamin C to regenerate vitamin E in a synergistic action to produce even much greater significant protection against lipid peroxidation. This might be due to pro-oxidant activity of vitamin C at certain dosages, depending on the presence of transition metal ions such as  $Fe^{3+}$  (Paolini *et al.*, 1999; Halliway *et al.*, 1999).

The increase in SOD activity seen in the reserpine-only treated group, could be a compensatory response to the OS resulting from the excessive production of free oxygen radicals. This is a first line of antioxidant defence against OS, scavenging  $O_2^{2-}$  in both cytosol and mitochondrial inter-membrane space (Rodriguez *et al.*, 2011). However, the activity of SOD in the vitamin groups was even significantly higher, probably due to augmentation of the endogenous antioxidant enzyme by the vitamins. Again, the co-administration did not show more efficacy probably due to the pro-oxidant effect of vitamin C. This finding is consistent with the work of Serra *et al.* (2001), but contrary to other more recent studies (Sunday *et al.*, 2014; Cracium *et al.*, 2016).

The significant increase in CAT activity across vitamin groups, with the greatest significance observed in the group given both vitamins is probably due to their synergistic effect against OS (Faria *et al.*, 2005; Khadkhodae *et al.*, 2008; Baptista-Ortega and Ruiz-Feria, 2010; Yarube and Ayo, 2011; Dawud *et al.*, 2014; Bursac-Metrovic *et al.*, 2016). CAT is part of the second line antioxidant defence, scavenging for  $H_2O_2$  generated by SOD activity intracellularly (Pillay and Yao, 2015). Hence, increase in SOD activity will lead to increase in  $H_2O_2$  generation, leading to a compensatory increase in CAT activity.

The mild increase in GSH level, although not statistically significant, may be due to a compensatory response to increase generation of free radicals, which is contrary to the work of Sian *et al.* (1994), that showed decrease in GSH level in the SNc of PD brains. Although expected, there was no appreciable increase in the GSH level in the groups treated with vitamins.

This may be due to an overwhelming level of OS and it's in line with the findings of Nayak *et al.* (2018), who also found no significant alteration in GSH level in rat brain exposed to aluminium and ethanol toxicity, following vitamin E supplementation for 4 weeks. Since GPx catalyses a reaction in which GSH detoxifies  $H_2O_2$  to water, the GSH level is expected to increase when there is decrease in the GPx activity, as seen in the present study. This is also in line with the findings of Sunday *et al.* (2014), who found decrease in GPx as a reciprocal to SOD activity, although other studies showed no significant alteration (Cracium *et al.*, 2016) in the GPX activity.

All the above findings showed a significant evidence of OS induced by reserpine in agreement with several other studies (Sanghavi *et al.*, 2010; Fernandez *et al.*, 2012; Eftimov *et al.*, 2014; Hsiang-Chien *et al.*, 2015; Dwivedi and Tomar, 2016). However, the administration of the antioxidants (vitamins C and E) was significantly protective against the OS, with the co-administration of the two antioxidants more potent than single vitamin administration. These findings of the present study on biomarkers of OS is contrary to other studies who showed generalised decrease in the SOD, CAT, GPx activities and GSH level (Napolitano *et al.*, 2011; Koppula *et al.*, 2012).

The beneficial effect of antioxidants and antioxidant enzymes is seen in scavenging of free radicals generated during OS and neuroprotection. These antioxidant systems prevent the generation and actions of ROS and provide a potential mechanism for ameliorating OS. Vitamin E, being a chain-breaking antioxidant within the lipid membrane (Kamal-Eldin and Appelqvist, 1996), protected against lipid peroxidation to a very significant extent as seen in this study and, it was itself regenerated by the vitamin C (Ambali *et al.*, 2010; Harrison, 2012), which is a powerful water-soluble antioxidant within the cytoplasm and outside the membrane, and may help to boost the endogenous antioxidant levels. The vitamin E in turn, probably played a role in the biosynthesis of more endogenous vitamin C (Machlin and Gabriel, 1980) to boost the antioxidant neuroprotective effect.

## CONCLUSION

From this study, co-administration of vitamins C and E was only partially neuroprotective against reserpine-induced OS in mice. Although, no significant neuroprotection was observed over the single vitamin administration, this finding depicts a great potential in slowing down the progression of NDDs like PD. Introducing such neuroprotective therapy, which are readily available and affordable, into the standard

treatment of PD will be highly beneficial. More studies are however needed, to explore these vitamins C and E at different dosages.

#### ACKNOWLEDGEMENT

No external source of funding obtained. We appreciate the contributions of Mr Emmanuel Solomon Nachamada to the success of this study.

#### REFERENCES

- Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, 105: 121-126.
- Ambali, S. F., Ayo, J. O., Ojo, S. A. and Esievo, K. A. (2010). Vitamin E protects wistar rats from chlorpyrifos-induced increase in erythrocyte osmotic fragility. *Food Chemistry and Toxicology*, 48(12): 3477-3480.
- Andersen, J. K. (2004). Oxidative stress in neurodegeneration: Cause or consequence? *Nature's Medicine*, 10: 18–25.
- Atawodi, S. E., Adekunle, O. O. and Bala, I. (2011). Antioxidant, organ protective and ameliorative properties of methanol extract of *Anogeissus leiocarpus* stem bark against carbon tetrachloride-induced liver injury. *International Journal of Pharmacological Sciences and Research*, 2(6): 1443-1448.
- Baptista-Ortega, J. and Ruiz-Feria, C. A. (2010). L-Arginine and antioxidant Vitamins E and C improve the cardiovascular performance of broiler chickens grown under chronic hypobaric hypoxia. *Poultry Science*, 89 (10): 2141-2146.
- Barreto, G. E., Larkov, A. and Moran, E. V. (2015). Beneficial effect of nicotine, cotinine and its metabolites as potential agents for Parkinson's disease. *Frontiers in Aging Neuroscience*, 6(340). doi: 10.3389/fnagi.2014.00340.
- Blesa, J. and Przedborski, S. (2014). Parkinson's Disease: animal models and dopaminergic cell vulnerability. *Frontiers in Neuroanatomy*. doi: 10.3389/fnana.2014.00155.
- Bursac-Metrovic, M., Milovanovic, D. R., Mitic, R., Jovanovic, D., Sovrljic, M., Vasiljevic, P., Tomovic, J. and Manojlovic, N. (2016). Effects of L-ascorbic acid and alpha-tocopherol on biochemical parameters of swimming-induced oxidative stress in serum of guinea pigs. *African Journal of Traditional, Complementary and Alternative Medicines*, 13(4): 29-33.
- Clark, C. E. (2007). Parkinson's disease. *British Medical Journal*, 335(7617): 441-445.
- Cracium, E. C., Dronca, E. and Leach, N. V. (2016). Antioxidant enzyme activity in subjects with Parkinson's disease under L-DOPA therapy. *Human and Veterinary Medicine Bioflux*, 8(2): 124-127.
- Czerska, M., Mikolajewska, K., Zielinski, M., Gromadzinska, J. and Wasowicz, W. (2015). Today's oxidative stress markers. *Medycyna Pracy*, 66(3): 393-405.
- Davie, C. A. (2008). A review of Parkinson's disease. *British Medical Bulletin*, 86: 109-127.
- Dawud, F. A., Mabrouk, M. A. and Umar, I. A. (2014). Effects of vitamins C and E on aspirin-induced gastric mucosal damage and oxidative stress. *Journal of Biological Sciences*. 6(1): 36-41.
- Dias, V., Junn, E. and Mouradian, M. M. (2013). The role of oxidative stress in Parkinson's disease. *Journal of Parkinson's Disease*, 3(4): 461-491.
- Dorsey, E. R. and Bloem, B. R. (2018). The Parkinson pandemic – a call to action. *Journal of American Medical Association - Neurology*, 75(1): 9-10.
- Dorsey, E. R., Sherer, T., Okun, M. S. and Bloem, B. R. (2018). The emerging evidence of the Parkinson pandemic. *Journal of Parkinson's Disease*, 8: 3-8.
- Dwivedi, G. and Tomar, V. (2016). Protective effects of ipomoea aquatic against reserpine-induced oxidative stress in brain using mice. *International Journal of Basic and Clinical Pharmacology*, 5(5): 2123-2129.
- Eckert, A., Keil, U., Marques, C. A., Bonert, A., Frey, C., Schussel, K. and Muller, W.E. (2003). Mitochondrial dysfunction, apoptotic cell death and Alzheimer's disease. *Biochemical Pharmacology*, 66: 1627-1634.
- Eftimov, M., Dobрева, C., Velkova, D. and Valcheva-Kuzmanova, S. (2014). Effect of *Aronia melanocarpa* fruit juice on reserpine-induced hypokinesia and oxidative stress in rats. *Scripta Scientifica Pharmaceutica*, 1(2): 29-34.
- Ellman, G. L. (1959). Tissue sulphhydryl groups. *Archives of Biochemistry and Biophysics*, 82: 70-77.
- Fahn, S. (1999). Parkinson disease, the effect of levodopa and the ELLDOPA trial (earlier vs later L-dopa). *Archives of Neurology*, 56: 529–535.
- Fahn, S. (2003). Description of Parkinson's disease as a clinical syndrome. *Annals of New York Academy of Science*, 991: 1–14.
- Faria, R. R., Abilo, V. C., Grassi, C., Chinen, C. C., Negrao, L. T. R., deCastro, J. P. M. V., Fukushima, D. F., Rodrigues, M. S. D., Gomes, P. H. Z., Registro, S., de Carvalho, R. C., DAlmeida, V., Silva, H. R., Ribeiro, R. A. and Frussa-Filho, R. (2005). Beneficial effects of vitamin c and vitamin e on reserpine-induced oral dyskinesia in rats: critical role of striatal catalase activity. *Neuropharmacology*, 48(7): 993-1001.

- Fernandez, V. S., Santos, J. R., Leao, A. H., Medeiros, A. M., Melo, T. G., Izidio, G. S., Cabral, A., Ribeiro, R. A., Abilio, V. C., Ribeiro, A. M. and Silva, R. H. (2012). Repeated treatment with a low dose of reserpine as a progressive model of Parkinson's disease. *Behavioural Brain Research*, 231(1): 154-163.
- Ferreira, S. and Winterer, G. (2009). Neuroprotective and neurotoxic effects of nicotine. *Pharmacopsychiatry*, 42(6): 255-265.
- Freitas, R. M., Vasconcelos, S. M. M., Souza, F. C. F., Viana, G. S. B. and Fonteles, M. M. F. (2005). Oxidative stress in the hippocampus after pilocarpine-induced status epilepticus in wistar rats. *The FEBS Journal*, 272(6): 1307-1312.
- Gagne, J.J., Power, M.C. (2010). Anti-inflammatory drugs and risk of Parkinson's disease. *Neurology*, 74(12): 995-1002.
- Harrison, F. E. (2012). A critical review of vitamin C for the prevention of age-related cognitive decline and Alzheimer's disease. *Journal of Alzheimer's Disease*, 29(4): 711-726.
- Hsiang-Chien, T., Mao-Hsien, W., Hung-Sheng, S., Yi, C., and Kuo-Chi, C. (2015). Epigallocatechin-3-gallate prevents the reserpine-induced impairment in short-term social memory in rats. *Behavioral Pharmacology*, 26(8): 741-747.
- Jenner, P. (2003). Oxidative stress in Parkinson's disease. *Annals of Neurology*, 53(3): 26-36
- Joghataie, M. T., Roghani, M., Negahdar, E. and Hashemi, L. (2004). Protective effect of caffeine against neurodegeneration in a model of Parkinson's disease in rat: behavioral and histochemical evidence. *Parkinsonism and Related Disorders*, 10(8): 465-468.
- Kamal-Eldin, A. and Appelqvist, L. A. (1996). The Chemistry and Antioxidant properties of tocopherols and tocotrienols. *Lipids*, 31:671-701.
- Khadkhodae, M., Khaster, H., Faghihi, M., Ghaznavi, R. and Zahmatkesh, M. (2008). Effects of co-supplementation of vitamins E and C on gentamycin-induced nephrotoxicity in rat. *Experimental Physiology*, 90(4), doi: 10.1113/expphysiol.2004.029728.
- Kohen, R. and Nyska, A. (2002). Oxidation of biological systems: oxidative stress phenomenon, antioxidants, redox reaction and methods for their quantification. *Toxicologic Pathology*, 30(6): 620-650.
- Koppula, S., Kumar, H., More, S. V., Kim, B. W., Kim, I. S. and Choi, D. K. (2012). Recent advances of the neuroprotective potentials of antioxidants in experimental models of Parkinson's disease. *International Journal of Molecular Sciences*, 13. doi:10.3390/ijms130810608
- Lees, J., Hardy, J. and Revesz, T. (2009). Parkinson's disease. *The Lancet*, 373 (9680): 2055-2066.
- Lin, M. T. and Beal, M. F. (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*, 443:787-795
- Machlin, L.J. and Gabriel, E. (1980). Interactions of vitamin E with vitamin C, vitamin B12 and zinc. *Annals of the New York Academy of Science*, 355(1): 98- doi: 10.1111/j.1749-6632.1980tb21330.x
- Mahadik, S. P., Evans, D. and Lal, h. (2001). Oxidative stress and role of antioxidant and omega-3 essential fatty acid supplementation in schizophrenia. *Progress in Neuropsychopharmacology, Biology and Psychiatry*, 25: 463-493.
- Misra, H. P. and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chemistry*, 247(10): 3170-3175.
- Miyake, Y., Fukushima, W., Tanaka, K., Sasaki, S., Kiyohara, C., Tsuboi, Y., Yamada, T., Oeda, T., Miki, T., Kawamura, N., Sakae, N., Fukuyama, H., Hirota, Y., Nagai, M. and Fukuoka K. (2011). Dietary intake of antioxidant vitamins and risk of Parkinson's disease: a case-control study in Japan. *European Journal of Neurology*, 18(1): 106-113.
- Mohamed, S., Jawis, N., Abass, S. and Krasilshchikov, O. (2015). Effects of dietary vitamins C and E supplementation on hepatic and renal function on young weightlifters. *Periodisation, British Journal of Sports Medicine*, 2(2): 24-29.
- Montine, K. S., Quinn, J. F., Zhang, J., Fessel, J. P., Roberts, L. J., Morrow, J. D. and Montine, T. J. (2004). Isoprostanes and related products of lipid peroxidation in neurodegenerative diseases. *Chemistry and Physics of Lipids*, 128: 117-124.
- Napolitano, A., Manini, P. and D'Ischia, M. (2011). Oxidation chemistry of catecholamines and neuronal degeneration: an update. *Current Medical Chemistry*, 18(12): 1832-1845.
- Nayak, P., Sharma, S. B. and Chowdary, N. V. S. (2018). Alpha-tocopherol supplementation restricts aluminium- and ethanol-induced oxidative damage in rat brain but fails to protect against neurobehavioral damage. *Journal of Dietary Supplements*, 10.1080/19390211.2018.1451940.
- Okhawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Annals of Biochemistry*, 95(2): 351-358.

- Ozsoy, O., Seval-Celik, Y., Hacioglu, G., Yargicoglu, P., Demir, R., Agar, A. and Aslan, M. (2011). The influence and the mechanism of docosahexanoic acid on a mouse model of Parkinson's disease. *Journal of Neurochemistry International*, 59(5): 664-670.
- Pillai, A. and Yao, J. K. (2015). Antioxidant interventions in neuropsychiatric disorders. In: Dietrich-Muszalska, A., Chauhan, V. and Grignon, S. (eds.). *Studies on Neuropsychiatric Disorders: Oxidative Stress in Applied Basic Research and Clinical Practice*. New York, NY: Humana Press, pp 505-520. doi: 10.1007/978-1-4939-0440-2\_25.
- Powers, S. K. and Jackson, M. J. (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiologic Review*, 88(4): 1243-1276.
- Rajagopalan, R., Kode, A., Penumatha, S. V., kallikat, N. R. and Venugopal, P.M. (2004). Comparative of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. *Journal of Pharmacology and Pharmaceutical Sciences*, 83: 2747-2752.
- Rodriguez, R. A., Wuwial, E., Perez, V. I., Lambert, A. J., Edrey, Y. H., Lewis, K. N., Grimes, K., Lindsey, M. L., Brand, M. D. and Buffenstein, R. (2011). Walking the oxidative stress tightrope: a perspective from the naked mole-rat, the longest living rodent. *Current Pharmaceutical Design*, 17: 2290-2307.
- Roghani, M. and Behzadi, G. (2001). Neuroprotective effect of vitamin E on the early model of Parkinson's disease in rat: behavioral and histochemical evidence. *Brain Research*, 891(1): 211-217.
- Saaksjarvi, K., Knekt, P., Rissanen, H., Laaksonen, M. A., Reunanen, A. and Mannisto, S. (2008). Prospective study of coffee consumption and risk of Parkinson's disease. *European Journal of Clinical Nutrition*, 62(7): 908-915.
- Sanghavi, C. R., Barhate, S. A., Mahajan, S. M., Mohan, M. and Kasture, S. B. (2010). Korean ginseng extract attenuates reserpine-induced orofacial dyskinesia and improves cognitive dysfunction in rats. 704-715. doi: <https://doi.org/10.1080/14786410802583031>.
- Sarmento-Silva, J. A., Lima, R. H., Cabral, A., Meurer, Y., Ribeiro, A. M. and Silva, R. H. (2014). Alpha-Tocopherol Counteracts Cognitive and Motor Deficits Induced by Repeated Treatment with Reserpine. *Biochemistry and Pharmacology (Los Angeles)* 3: 153. doi:10.4172/2167-0501.1000153
- Schapira, A. H. V. (2005). Present and future drug treatment for Parkinson's disease. *Journal of Neurol. Neurosurg. and Psychiatry*, 76: 1472-1478.
- Serra, J. A., Dominguez, R. O., de Lustig, E. S., Guareschi, E. M., Famulari, A. L., Bartolome, E. L., Marschoff, E. R. (2001). Parkinson's disease is associated with oxidative stress: comparison of peripheral antioxidant profiles in living Parkinson's, Alzheimer's and vascular dementia patients. *Journal of Neurologic Transmission*, 108(10): 1135-1348
- Sian, J., Dexter, D. T., Less, A. J., Daniel, S., Jenner, R. and Marsden, C. D. (1994). Glutathione-related enzymes in brain in Parkinson's disease. *Annals of Neurology*, 36: 356-361.
- Sunday, O. P., Adekunle, M. F., Temitope, O. T., Richard, A. A., Samuel, A. A., Olufunminyi, A. J. and Elizabeth, O. O. (2014). Alteration in antioxidants level and lipid peroxidation of patients with neurodegenerative diseases {Alzheimer's disease and Parkinson disease}. *International Journal of Nutrition, Pharmacology and Neurological Diseases*, 4(3): 146-152.
- Tanko, I., Benjamin, N., Yahuza, F., Jimoh, A., Mohammed, K.A., Muhammad, A., Yerima, M. And Mohammed, A. (2014). Ameliorative effect of l-ascorbic acid and  $\alpha$ -tocopherol co-administration on blood glucose levels and some haematological indices on high fed fat diet-induced diabetes on wistar rats. *Nigerian Journal of Scientific Research*, 13(2): 26-31.
- Tanner, C. M. and Aston, D. A. (2000). Epidemiology of Parkinson's disease and akinetic syndromes. *Current Opinions in Neurology*, 13: 427-430.
- Thomas, L. (2017). Parkinson's Disease Tremor. Retrieved on 13<sup>th</sup> March, 2018 from <https://www.news-medical.net/health/Parkinsons28099s-disease-tremor.aspx>
- Wahner, A. D., Bronstein, J. M., Bordelon, Y. M. and Ritz, B. (2007). Non-steroidal anti-inflammatory drugs may protect against Parkinson's disease. *Neurology*, 69(19): 1836-1842.
- Weinreb, O., Amib, T., Bar-Am, O. and Youdim, M. (2010). Rasagiline: a novel antiparkinsonian monoamine oxidase-B inhibitor with neuroprotective activity. *Progress in Neurobiology*, 92:330-344.
- Yarube, I. U. and Ayo, J. O. (2011). Modulatory role of antioxidant vitamins C and E on erythrocyte osmotic fragility induced by chronic sodium nitrate administration in rats. *Bayero Journal of Pure and Applied Sciences*, 4 (1): 115-120