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Co-Administration of Vitamins C and E is Protective against Reserpine-induced Motor Impairment in Mice.

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Abstract

Background: The conventional treatments for Parkinson's disease, the most common movement disorder globally, have not been able to halt its progression hence, newer approaches targeting its pathogenesis are being explored. We investigated the effect of vitamins C and E (in combination) on reserpine-induced motor impairment in mice.

Materials and Methods: Twenty-five mice were randomly grouped into five groups of five mice each. All the groups except group I (control), were given an alternate days injection of reserpine 0.1 mg/kg intraperitoneally. Groups III and IV were administered vitamin E 200 mg/kg/day (vitamin E group), and vitamin C 250 mg/kg/day (vitamin C group), respectively while group V (co-administered group) was given both vitamins orally. Group II (reserpine group) received nothing in addition to reserpine. All drugs were given concurrently for 28 days. The neurobehavioral assessment was performed using beam walking and open field tasks. Results were presented as mean±SEM and statistical significance was placed at $p < 0.05$.

Results: There were significant increases in a number of foot slips (3.60 ± 0.68 ; $p = 0.002$) and the time spent in reaching the 'safe' platform (36.60 ± 5.78 s; $p = 0.0001$) in the reserpine group, both of which were markedly reduced in the co-administered group (0.25 ± 0.25 and 3.00 ± 0.41 s respectively). The co-administered group demonstrated a marked decrease in transfer latency (10.33 ± 1.45 s; $p = 0.005$) and crossed significantly more lines (56.00 ± 13.53 lines; $p = 0.0001$) in the open field compared to the reserpine group (214.00 ± 64.16 s and 4.3 ± 1.67 lines respectively).

Conclusion: Co-administration of vitamins C and E protected against motor impairment induced by reserpine in mice.

Keywords: co-administration, motor impairment, neuroprotective, reserpine-induced, vitamins

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease as well as the most common movement disorder worldwide (1-3). As one of the fastest-growing neurological disorders, PD is a leading cause of disability worldwide (4). It affects about ten million people aged 65 years and above (3), and from projections, this prevalence will be doubled by 2040 due to increasing life expectancy and industrialization (5).

Several treatment modalities exist for PD among which includes the gold-standard levodopa, dopamine agonists and monoamine oxidase B inhibitors among others (6-10). Other therapies such as non-steroidal anti-inflammatory drugs (NSAIDs) (11), nicotine (12), caffeine (13-14), docosahexaenoic acid (15) and exercises (16-18), have also been proposed. However, these therapies are efficient at controlling the motor symptoms only for a couple of years when introduced early in the disease process and are associated with side effects (19). In addition, none of these therapies slows down or halts the disease progression (20). These have shifted the focus of research to the use of antioxidants, given the central role oxidative stress plays in the pathogenesis of most neurodegenerative diseases (NDD) including PD (21).

Vitamins C and E protect cells against oxidative damage by neutralizing free radicals. Both vitamins have protective properties as individual antioxidants (22-24) but combining them was shown to produce greater effects against oxidative stress and nephrotoxicity (25), as well as hyperglycemia (26) in rats. The co-administration of vitamins C and E also potentiated the effect of each other in reserpine-induced oral dyskinesia in rodents (27), suggesting a complete antioxidant defense (28).

While vitamin C plays a role in recycling of vitamin E (24, 29), vitamin E in turn, is involved in the biosynthesis of vitamin C (30, 31). Therefore, the combination of the two vitamins may enhance their antioxidant potentials due to this synergistic interaction as reported in some studies (32-35). We, therefore, aimed to investigate the effects of co-administration of vitamins C and E on reserpine-induced motor impairment in mice using

beam-walking and open field tasks.

Materials and Methods

Materials

Vitamins C (Catalog number: HY-B0166/CS-2006; Lot number: 16468) and E (Catalog number: HY-B1278A/CS-7804; Lot number: 31618) as well as reserpine (Catalog number: HY-N0480/CS-1913; Lot number: 27769) were purchased from MedChem Express, U.S.A. Glacial acetic acid was purchased from BDH Chemicals Ltd., Poole, England (Lot number: 27103).

Ethical Approval

This study was approved by the Ahmadu Bello University Committee on Animals Use and Care (approval number: ABUCAUC/2020/72).

Experimental Animals

A total of 25 male mice (*Mus musculus*), 8-12 weeks old and weighing 20-40g were used for this study due to their genetic, biological and behavioral similarities with humans and they are the most commonly used strains in neuropathologic researches. They were obtained from the Department of Human Physiology, Ahmadu Bello University (ABU), Zaria. They were kept according to their groups in plastic cages with softwood shavings, which were changed every other day and allowed free access to mice chow and water under standard laboratory conditions. They were all drug- and test-naïve and certified healthy.

Drug formulation and route of administration

The reserpine was constituted with glacial acetic acid (made up to 2.5%). It was given at 1 ml/kg intraperitoneally due to its poor oral absorption and low bio-availability. Vitamin C was dissolved in distilled water and given at a dose of 10 ml/kg orally. Vitamin E was dissolved in 0.1% dimethyl sulfoxide (DMSO), which was purchased from Guangdong Guanghua Sci-tech Co. Ltd, China (Catalog number: 20170215) and administered at a dose of 2 ml/kg orally.

Induction of Parkinsonism

An alternate day's administration of reserpine injection at 0.1mg/kg *i.p.* over 4 weeks was used to closely mimic the slowly progressive neurodegenerative process of PD in the mice (36). Features such as rigidity, hypokinesia or akinesia were suggestive of movement disorders in the mice.

The beam walking task

The beam walking test was performed 24 hours after the last reserpine administration according to the method described by Stanley *et al.* (38). The beam consists of 80 cm long and 1 cm wide, horizontal, cylindrical rod suspended about 65cm above a table with one end attached to a 'safe' platform (13 x 20 x 27 cm). There was an opened cage on the table directly below the beam, containing soft foams to avoid injury in case of a fall. Each mouse was placed at the other end and was expected to walk towards the safe platform without stopping or stalling. However, when they fail to proceed forward, a gentle prodding or pushing from behind was given to encourage the mouse to continue moving. During the training session, each mouse was entitled to 1-3 trials. Once in the safe platform, each mouse was allowed about 10-15 seconds to habituate itself, then returned to its home cage to rest for about 10 minutes. During this interval, 3-4 mice were trained to make efficient use of time.

In the testing phase, each mouse was entitled to 1-2 trials with little or no interference. The number of foot slips, as well as the time it takes to move from the starting point to the safe platform, was recorded with a camera. A foot slip is defined as when at least one of the feet comes off the beam or the mouse hind limbs completely lose grip of the beam, which may lead to a fall. It was ensured that the beam was cleaned off mouse droppings after each trial with cotton wool soaked in 5% alcohol and allowed to dry before the next trial.

Open field test (OFT)

The OFT is a common method for exploratory behavior and general activity in rodents (39). It was carried out according to the method described by Eftimovet *et al.* (40). The open field is a wooden, square arena (72 x 72 cm), 36cm high and 20cm elevated above the ground surface. The floor is made of transparent glass with white paper placed directly underneath. The paper is equally divided into 16 large squares by bold black ink.

A mouse was placed into the first square at one of the four corners of the open field. It is expected that the mouse leaves the square to explore the entire arena as a result of natural curiosity. They were observed for five minutes. The transfer latency (in seconds) to cross the first line, as well as the number of lines, crossed at the end of the session was recorded and analyzed with the aid of an overhead camera. After 5 minutes, the mouse

Group I:	No intervention was given throughout the study period. This group served as normal control.
Group II	reserpine (0.1mg/kg) intraperitoneally (<i>i.p.</i>) on alternate days (3d) over 4 weeks. They served as the reserpine group
Group II:	with vitamin E (200mg/kg/day) orally (37) plus reserpine (0.1mg/kg) <i>i.p.</i> on alternate days over 4 weeks.
Group IV	vitamin C (250mg/kg/day) orally (37) plus reserpine (0.1mg/kg) <i>i.p.</i> on alternate days over 4 weeks.
Group V	vitamin C (250mg/kg/day) orally and vitamin E (200mg/kg/day) orally (37) plus reserpine (0.1mg/kg) <i>i.p.</i> on alternate days over 4 weeks.

was returned to its home cage and the entire arena was cleaned with cotton wool soaked in 5% ethanol solution and allowed to dry before the next mouse is introduced.

Figure 1: Mice during an Open Field Test. (adopted by the authors in this study)

Statistical Analysis

Data were expressed as mean $\pm$ standard error of the mean (SEM) and analyzed using one-way analysis of variance (ANOVA), followed by *Tukey's* posthoc test to compare differences in the mean between groups. Values at $p < 0.05$ were considered statistically significant using SPSS version 23.0 (Armonk, NY: IBM Inc., USA, 2018).

Results

There was a significant increase ($p = 0.002$) in the number of foot slips observed in the reserpine group (3.60 ± 0.68) compared to the normal control. This was reduced by the administration of vitamins C (1.25 ± 0.63) and E (0.50 ± 0.29), and both vitamins (0.25 ± 0.25) at $p = 0.002$ (figure 2).

Figure 2: Number of foot slips by group while walking on the beam ($F = 6.584$, $p = 0.002$). Superscripts ^a and ^b indicate statistically significant difference compared to reserpine and normal control groups respectively (Control = normal control; Res = Reserpine; Vit= vitamin).

The time taken to reach the safe platform was significantly lessened ($p = 0.0001$) by vitamin E (5.50 ± 1.85 s), vitamin C (8.00 ± 1.22 s) and the co-administration of vitamins C and E (3.00 ± 0.41 s), compared to the reserpine group (36.60 ± 5.78 s) at $p < 0.001$ (table 1).

The latency to leave the first square of the open field in the reserpine-only treated group was significantly ($p = 0.005$) increased (214.0 ± 64.16 s) compared to the normal control. This was ameliorated by the adminis-

Table 1: Time taken to reach the safe platform by group on the beam-walking test

Groups	Time to reach the platform (s)
Normal Control	8.60 $\pm$ 1.81 ^a
Res only (0.1 mg/kg)	36.60 $\pm$ 5.78 ^b
Res+Vit E (200 mg/kg)	5.50 $\pm$ 1.85 ^a
Res+Vit C (250 mg/kg)	8.00 $\pm$ 1.22 ^a
Res+Vit C+Vit E	3.00 $\pm$ 0.41 ^a

Superscripts ^a and ^b indicate statistically significant difference compared to reserpine and normal control groups respectively ($F = 19.262$, $p = 0.000$. Control = normal control; Res = Reserpine; Vit= vitamin).

tration of vitamins C (116.7 ± 43.71 s), E (1.0 ± 0.00 s) and both vitamins (10.3 ± 1.45 s) at $p = 0.005$ (table 2).

Table 2: Transfer latency of mice to leave the starting square on the open field

Groups	Transfer Latency (s)
Control	1.00 $\pm$ 0.00 ^a
Res only (0.1 mg/kg)	214.00 $\pm$ 64.16 ^b
Res+Vit E (200 mg/kg)	1.00 $\pm$ 0.00 ^a
Res+Vit C (250 mg/kg)	116.67 $\pm$ 43.71 ^b
Res+Vit C+Vit E	10.33 $\pm$ 1.45 ^{ab}

Superscripts ^a and ^b indicate statistically significant difference compared to normal control and reserpine groups respectively ($F = 7.461$; $p = 0.005$. Control = normal control; Res = Reserpine; Vit= vitamin).

The co-administration of both vitamins C and E significantly increased ($p = 0.0001$) the number of lines crossed in the open field (56.0 ± 13.53), more than that produced by vitamin E (52.3 ± 5.69) and vitamin C (19.7 ± 4.63) as a single administration. However, a significant decrease was noted in the reserpine group (4.3 ± 1.67) compared to the control (102.3 ± 9.21) at $p < 0.001$ (figure 3).

Figure 3: Number of lines crossed by groups on the open field ($F = 22.110$, $p = 0.000$). Superscripts ^a and ^b indicate statistically significant difference compared to control and reserpine only treated groups respectively (Control = normal control; Res= Reserpine; Vit= vitamin).

Discussion

Movement disorders such as akinesia/dyskinesia, rigidity, tremors and postural instability are the cardinal motor symptoms of PD clinically required as part of the diagnostic criteria (41). From our result, there was a significant delay to reach the safe platform at the end of the beam with a corresponding increase in the number of foot slips in the reserpine group compared to the normal control. However, these were significantly

ameliorated by the pre-treatment with vitamins C and E as single administration as well as co-administration, and the greatest effect was noted in the co-administered group. These findings explain the akinesia and rigidity associated with dopamine depletion in PD. The difficulty in initiating voluntary movements observed in the reserpine group was very striking. This is what is referred to as dyskinesia, a cardinal feature of PD. It could be very distressing to the extent that the highest degree of concentration must be exerted to perform even the simplest movement. Even when movements do occur, they are usually met with stiff resistance and staccato in character instead of smooth.

There was also a significant latency in the reserpine group to cross the first line of the open field, which was significantly ameliorated by all the vitamin groups, with the greatest significance seen in the vitamin E treated group. Although not assessed in this study, this may be due to the ability of vitamin E to protect dopaminergic neurons against OS and degeneration (22). This finding agrees with the work of Dwivedi and Tomar (42), which also showed significantly severe hypokinesia both in terms of latency to leave the starting square of the OFT and the number of lines crossed. However, Nayak *et al.* (43) demonstrated the failure of vitamin E to protect against neurodegeneration in rat brains at a given dose, making the rats perform poorly in some motor coordination tasks. There was also a significant increase in the number of lines crossed following the co-administration of both vitamins compared to the reserpine group, and this effect was more profound than that observed following single administrations. This is probably due to the synergistic effects between vitamins C and E in neuroprotection as revealed by a number of other studies (32, 33).

The above findings showed significant motor impairment induced by reserpine, which is in line with several studies (44-46). However, we demonstrated the neuroprotective efficacy of the antioxidants (vitamins C and E) against this impairment. This was more evidenced following the co-administration of the vitamins probably due to the synergism in their actions (34, 35), producing a complete antioxidant defense.

Conclusion

In this study, vitamins C and E produce significant amelioration of reserpine-induced motor impairment in mice, with a superior effect following co-administration of both vitamins compared to single vitamin administration. This suggests a great potential in slowing down a neurodegenerative process in NDDs like PD.

However, this study is not without limitations such as possible modulation of vesicular monoamine transporter 2 (VMAT-2) expression by these vitamins; their histological effects on the dopamine neurons in targeted areas of the brain; their effects on dopamine neurotransmitter itself together with other catecholamines in different brain regions; effects of these vitamins

at different doses; etc. Therefore, future researches should be focused on these directions.

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