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Neuroprotective Role of Green Tea Polyphenols on the Superior Colliculus in MPTP Mice Model of Parkinson's Disease

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Authors' contributions

This work was carried out in collaboration between all authors. Author PDS designed the study and wrote the protocol and the final draft of the manuscript. Author KAK managed the literature searches and wrote the first draft of the manuscript. Authors PDS, ADA and KAK managed the laboratory animals. Authors KAK, OFS and ADA performed the laboratory analyses. Author OFS performed the statistical analysis. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aim: This study investigated the neuroprotective role of green tea polyphenols on the superior colliculus of MPTP mice model of Parkinson's disease.
Study Design: Twenty-five adult male mice (*Mus musculus)* weighing between 20-30 grams were used for this study. The mice were randomly placed into five groups of five mice each: A (Control; mice pellets), B (MPTP 10mg/kg, IP), C (MPTP + GT; 300 mg/kg GT orally), D (GT + MPTP), E (GT; 300 mg/kg).
Place and Duration of the Study: Department of Anatomy, Babcock University. Between February and May 2016.

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Methodology: The brains were removed weighed and the midbrain excised processed histology and stained routinely with H&E and silver stains.
Results: The results showed significant (P<0.005) reduction in the relative brain to body weight and neuronal density in the superior colliculus; characterized by neuronal atrophy as signaled by vacuolations and pyknosis in the MPTP and green tea only groups. Whereas pre-treatment with green tea polyphenol resulted in significant (P<0.005) increase in the relative brain to body weight and increase in the superior colliculus neuronal density when compared with the MPTP and green tea only groups.
Conclusion: From our results we can conclude that pre-treatment with green tea polyphenols confers greater protection on the superior colliculus in MPTP mice model of Parkinson's disease.

Keywords: Parkinson's disease; superior colliculus; MPTP; green tea polyphenol.

1. INTRODUCTION

Parkinson's disease (PD) is classically characterized by cardinal motor signs such as bradykinesia, muscular rigidity, tremor and postural instability. In addition, other non-motor symptoms have been reported, such as cognitive and perceptual changes. The perceptual changes reported include visual, olfactory, auditory and somatosensory impairment which is a direct reflection of disturbances in higher order processes affecting these basic sensory systems [1]. Other research reported that the massive burden of prioritizing visual stimuli from the environment lies with the superior colliculus [2]. This part of the brain may provide clues regarding the mechanisms leading to Parkinson's disease. The superior colliculus is a layered structure with the superficial layers receiving primary inputs from the retina and the deeper layers involved with subconscious guidance of eye movements and communicate the same information to other brain regions via corollary discharges to guarantee stability of the visual eye field [3,4]. The superior colliculus also receives numerous feedback stimuli from other brain regions such as basal ganglia and the frontal visual eye fields [5]. Parkinson's disease is a progressive, degenerative disorder, resulting from the loss of dopaminergic neurons in the substantia nigra, and presently has no cure. While the current treatments for Parkinson's are associated with serious side effects [6]. In the 1980s, PD prevalence was found to be low in Asian countries when compared to Europe and North America, which had significantly higher rates [7,8]. Apart from genetic factors, dietary habits like green tea consumption, which is more consumed by the Chinese population when compared to Caucasian, could explain this attribute [9 and 10]. Due to this possible link, in recent years, there were more studies devoted to exploring the effects of tea consumption on PD risk. Three case–control studies (in the US, Hong Kong and Singapore) and a cohort study of male health professionals in the US have all reported an inverse association between tea drinking and PD risk [11-13]. One study found such an effect for men but not for women [14]. On the other hand, a hospital based case-control study in France reported tea consumption to be a paradoxically risk factor for PD [15].

Considering the popularity of green tea beverages worldwide, there is enormous public interest in the health effects of its consumption.From the above perspective, Parkinson's disease might actually be a unique opportunity to study the role of green tea polyphenol on the superior colliculus.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Twenty-five adult male mice (*Mus musculus)* weighing between 20-30 g were used for this study. The animals were housed in clean plastic cages, well ventilated environment with temperature ranging between 24-28ºC in 12 hours light and 12 hours dark cycle. The animals were given standard mice pellets and water *ad libitum*, and were allowed to acclimatize for four weeks before commencing the experimental protocols. MPTP was bought from Adooq Bioscience, while Lindberg Standardized Green Tea Extract purchased from Nutrition Express, CA, USA was used for the study. Each capsule contained 500 mg of decaffeinated green tea extract standardized to contain 200 mg of EGCG, 95% polyphenols, 75% catechins, 40% EGCG. The extract was dissolved in distilled water to obtain a concentration of 300 mg/kg (300 mg/1000 g) body weight of the animal. While MRM Vegetarian Quercetin Extract was also used in the study to supplement the GTE and

increase its bioavailability. It was purchased from Nutrition Express Torrance, CA, USA. Each capsule standardized to contain 500mg of QU995 (The world's purest Quercetin) which ensures superior bioavailability. The capsule was dissolved in distilled water to obtain a concentration of 6mg/kg (1:5; Quercetin: GTE) [16].

The institutional committee on Animal Care and Use in Research, Education and Testing (ACURET) approval (16/BU-ACURET 45) was obtained and the animal experiments were conducted according to the NIH Guide on Laboratory Animals for Biomedical Research (NIH, 1978) and ethical guidelines for investigation of experimental pain in conscious animals [17].

2.2 Experimental Design

Following the four weeks of acclimatization, the animals were randomly divided into five (5) groups of five (5) animals each as follows:

- Group A: (Control Group) Mice were given dry food pellet and clean water *ad libitum.*
- Group B: (Negative Control Group) Mice were given 10 mg/kg of 1Methyl -4-phenyl-2,3,6-tetrahydropyridine (MPTP) per body weight intraperitoneally for 2 consecutive days; four times per day with two hour intervals [18].
- Group C: (Curative Group) Mice were given 10mg/kg of MPTP per body weight intraperitoneally for 2 consecutive days followed by a seven (7) day oral treatment with 300 mg/kg of Green Tea Extract supplemented with 6 mg/kg of Quercetin (GT).
- Group D: (Protective Group) Mice were given 300 mg/kg of Green Tea Extract orally supplemented with 6 mg/kg of body weight of Quercetin for seven (7) days consecutively followed by a two (2) day administration of 10 mg/kg of MPTP intraperitoneally four (4) times per day with a\ two (2) hour intervals.
- Group E: (Treatment group) Mice were given 300 mg/kg of Green Tea Extract supplemented with 6 mg/kg of body weight of Quercetin orally for 7 consecutive days.

2.3 Tissue Sample Preparation

At the end of four weeks the mice were euthanized by administering 10 g/kg body weight of Pentobarbital. The mice brains were carefully dissected out and weighed, some were fixed in 10% formal-saline for routine histological procedures.

2.4 Preparation of Histological Slides

Tissue preparation was carried out using the conventional paraffin embedding method. Tissue sections were stained with Haematoxylin and Eosin (H&E) to determine the general morphology [19] and Silver stain to show neuronal processes [20].

2.5 Photomicrography

Photomicrographs were taken using Omax led digital Microscope.

2.6 Cell Count

The neuronal cell count was done using image j software.

2.7 Statistical Analysis

Data were analysed by comparing values for different treatment groups with the values for individual controls using analysis of variance (ANOVA). Results were expressed as mean ± SE. The significant differences among values were analysed using Graph Pad version 7 at *P-value* = 0.05.

3. RESULTS

Fig. 1 shows the mean ± SE of relative brain weight of groups A (control) (1.540 ± 0.060), B (MPTP) (1.295 ± 0.025), C (MPTP +GT) (1.345 ± 0.075), D (GT+ MPTP) (1.375 ± 0.035), E (GT) (1.555 ± 0.035). The results of the one way ANOVA showed a significant effect of the treatments on the relative organ weight of the brain ($F_{(4, 5)}$ =537.3, $p<0.0001$), while Tukey's multiple comparisons test at $\alpha < 0.05$ showed significant weight decrease between the Control (1.540 ± 0.060) versus MPTP(1.295 ± 0.025); Control (1.540 ± 0.060) versus MPTP +GT (1.345 ± 0.075)and Control (1.540 ± 0.060) versus GT + MPTP(1.375 ± 0.035). Significance differences were also observed between the MPTP group and the intervention groups (GT+ MPTP and GT+ MPTP).

4. DISCUSSION

The superior colliculus is not only responsible for subconscious guidance of eye movements, it

also informs several brain regions about the on-going eye movement [2,3]. Corollary discharges from the superior colliculus to different brain regions are essential to guarantee the stability of the visual field. Many studies have been conducted without a specific focus on superior colliculus in patients with Parkinson's disease. Therefore this study was designed to investigate the role of green tea polyphenol in MPTP model of Parkinson's disease on this unique intracranial visual relay centre; The superior colliculus.

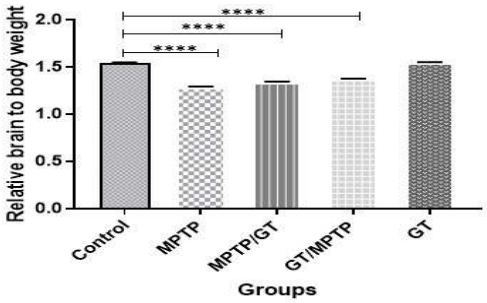

Fig. 1. Graph showing the mean relative brain weight

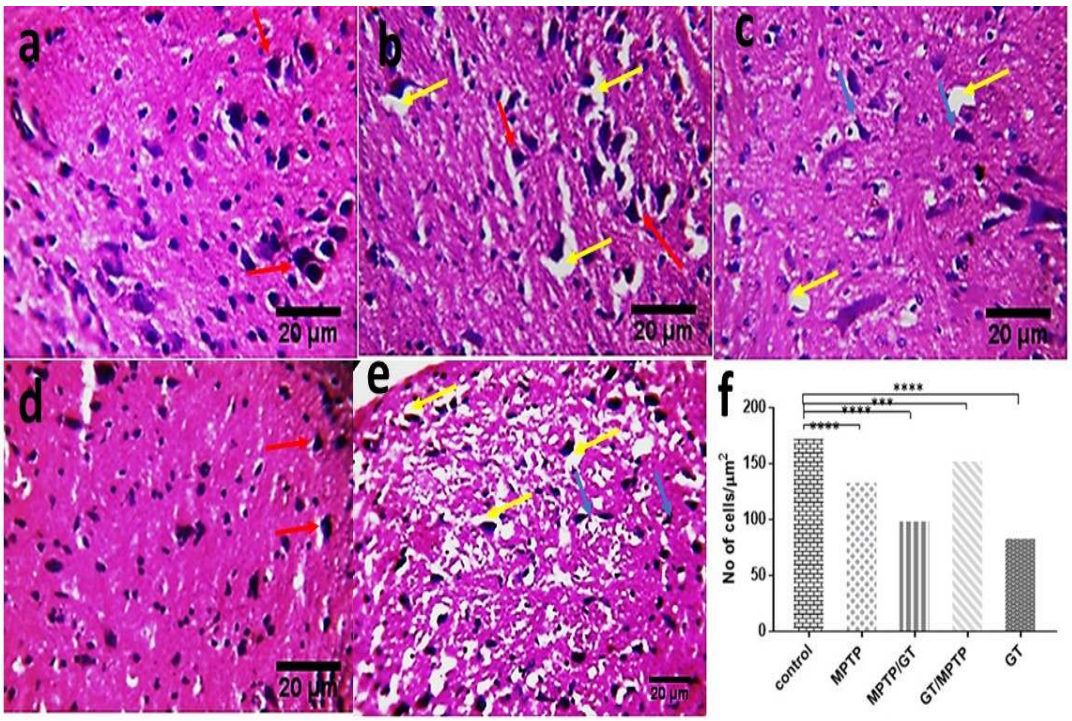

Plate 1. Showing the photomicrographs of mice superior colliculus stained with H&E X400: (a) Superior colliculus of control group showing neuronal cell bodies (red arrow). (b) Superior colliculus of MPTP treated mice with vacuolated neurons (yellow arrows). (c) Superior colliculus of MPTP+GT treated mice showing severe signs of necrosis characterised by vacuolations (yellow arrows) and pyknosis (blue arrows). (d) Superior colliculus of GT+MPTP treated mice with relatively preserved neuronal architecture. (e) Superior colliculus of GT treated mice showing severe signs of necrosis characterised by vacuolations (yellow arrows) and pyknosis (blue arrows). (f) Bar diagram showing the mean cell count in the superior colliculus of experimental groups; groups A (control) (174 ±2.8), B (MPTP) (136±2.8), C (MPTP +GT) (100 ± 2.8), D (GT+ MPTP) (154 ± 2.4), E (GT) (86 ± 2.6). The results of the one way ANOVA showed a significant effect of the treatments on the neuronal cell count of the superior colliculus ($F_{(4, 10)}$ =338.1, p<0.0001), while Tukey's multiple comparisons test at α < 0.05 showed significant increase in the neuronal cell count between the Control (174 ±2.8) versus MPTP (136±2.8); Control (174 ± 6.8) versus MPTP + GT (100 ± 2.4); Control (174 ± 6.8) versus GT + MPTP (154 ± 2.4) and Control (174 ± 6.8) versus GT (86 ± 2.4). Significance differences were also observed between the MPTP group and the intervention groups (GT+ MPTP and GT+ MPTP)

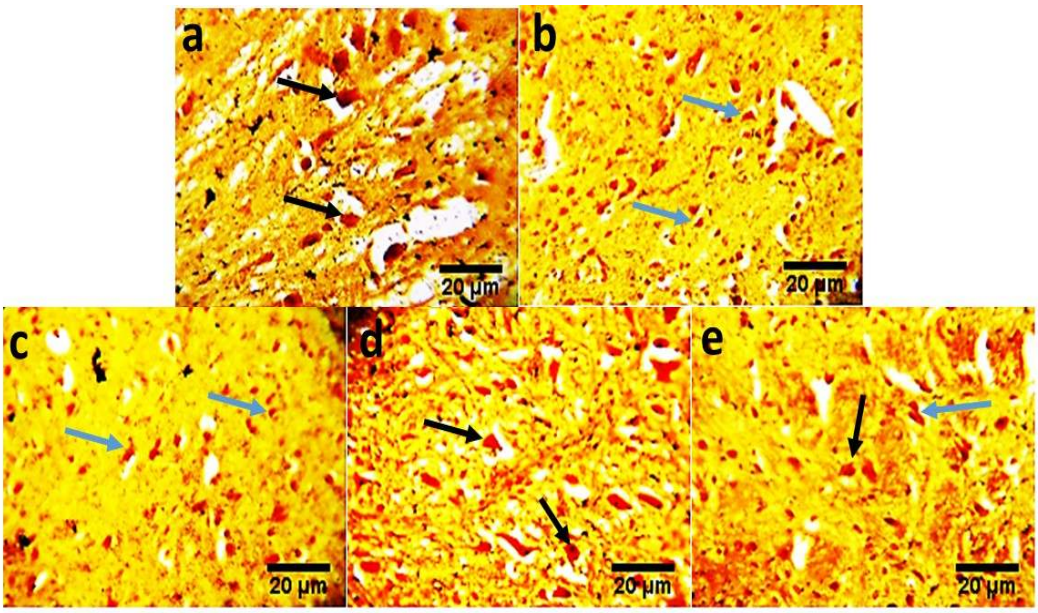

Plate 2. Showing the photomicrographs of mice superior colliculus Silver stained X400: (a) Superior colliculus of control group showing neuronal cell bodies (black arrows). (b) Superior colliculus of MPTP treated mice with severe signs of necrosis characterised pyknotic neurons (blue arrows). (c) Superior colliculus of MPTP+GT treated mice showing severe signs of necrosis characterised pyknosis (blue arrows). (d) Superior colliculus of GT+MPTP treated mice with relatively preserved neuronal architecture. (e) Superior colliculus of GT treated mice showing mild signs of necrosis characterised by pyknosis (blue arrows)

Our results showed that Parkinson disease in mice is characterised by reduction in the relative weight of the brain which in part reflect neuronal atrophy and degeneration in the superior colliculus as evidenced by pyknosis and vacuolations of neurons [Plate 1, Fig. b and Plate 2, Fig. b] whereas consumption of green tea polyphenol before induction of Parkinson's disease confers greater protection against the adverse effect of the disease [Plate 1, Fig. D and Plate 2, Fig. D]. These findings are further amplified by the significant decrease in neuronal cell count in the Parkinson's disease mice and corresponding significant preservation of neurones in the green tea pre-treated group [Plate 1, Fig. F]. Atrophy as seen in the MPTP group in this study may not only be the consequence of local Lewy body pathology as predicted by Braak's hypothesis on ascending degeneration in Parkinson's disease, but also a consequence of pathological deactivation, during or before visual perception [21] and, more fundamentally, as part of malfunctioning of the default mode network [22,23]. EGCG reduced neuronal cell death and induced nitric oxide synthase (NOS) expression in an MPTP mouse model of PD, thus providing further evidence for its neuroprotection via NO reduction [24]. Oral pre-treatment with EGCG prevented dopaminergic neuron loss in MPTP-treated mice [25]. With regards to specific tea polyphenols, the most important plausible mechanisms cited that may be exhibiting neuroprotective effects in PD are: (i) anti-oxidant and anti-chelating activities; (ii) inhibition of 'S aggregation; and (iii) modulation of cell signalling pathways [26-28] Since iron burden and ROS are attributed to neuronal damage, iron chelation and antioxidative strategies are useful to protect neurodegeneration. (-)-Epigallocatechin-3-gallate (EGCG) is an antioxidant used to counteract neurotoxin and oxidative damage due to its iron chelation and free radical scavenging capabilities. EGCG can induce the expression of several antioxidant enzymes, and eliminate ROS and electrophile generation in the progression of neurodegeneration. EGCG has also been shown to inhibit the inflammatory reactions against cancer and neurological diseases [29]. The implication of our results is that in untreated patients with PD the loss of dopaminergic neurons in the substantia nigra may be expected to result in increased inhibition of the superior colliculus with the resultant reduction of reflexive

saccades to irrelevant stimuli. On the other hand our results also showed that treating healthy mice with green with caused severe neuronal structural derangements [see Plate 1e and f] in the superior colliculus with corresponding functional consequences. This finding could be attributed to the dosage of green tea consumed. Previous works suggested that consumption of EGCG at high doses may promote, rather than attenuate, the inflammatory response in healthy adult mice [30]. Another study observed increased tumorogenesis by EGCG. In that study, the authors found that in an azoxymethane-induced tumor model, rats fed a high dose of green tea extract (3600 ppm) for 43 weeks had significantly higher tumor multiplicity, while those on a low dose (360 ppm) showed no difference compared to the control animals [31].

In summary it can be argued that far from being a bystander in the aetiology of PD, the superior colliculus has the massive burden of prioritizing visual stimuli from our environment and therefore functions as a pivotal 'gate keeper' to and from the basal ganglia and the frontal eye fields.

5. CONCLUSION

Based on our results, we can therefore conclude that pre-treatment with green tea polyphenols confers greater protection on the superior colliculus in MPTP mice model of Parkinson's disease via its beneficial biological effects as an antioxidant used to counteract neurotoxin and oxidative damage due to its iron chelation and free radical scavenging capabilities. However given the sharp contrast of our findings between the neuroprotective role of green tea in the diseased mice and the pro-inflammatory role of green tea in healthy mice; caution should be the watch word in recommending the consumption of green tea.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this paper and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Flowers KA, Robertson C. Perceptual abnormalities in Parkinson's disease: Top-down or bottom-up processes? Perception. 1995;24:1201-1221.
2. Andreas A Kardamakis‡, Juan Pe ´rez-Ferna ´ndez‡, Sten Grillner. Spatiotemporal interplay between multisensory excitation and recruited inhibition in the lamprey optic tectum. eLife. 2016;5:e16472.
3. Schiller PH. The role of the monkey superior colliculus in eye movement and vision. Invest Ophthalmol. 1972;11: 451–60.
4. Wurtz RH, Goldberg ME. The primate superior colliculus and the shift of visual attention. Invest Ophthalmol. 1972;11:441-50.
5. McHaffie JG, Stanford TR, Stein BE, Coizet V, Redgrave P. Subcortical loops through the basal ganglia. Trends Neurosci. 2005;28:401–7.
6. Shuhong Guo, Jingqi Yan, Tangbin Yang, Xianqiang Yang, Erwan Bezard, Baolu Zhao. Protective effects of green tea polyphenols in the 6-OHDA rat model of parkinson's disease. Through inhibition of ROS-NO pathway. Biological Psychiatry. 2007;62(12):1353–1362.
7. Li S, Schoenberg BS, Wang CC, Cheng X-M, Rui DY, Bolis CL, Schoenberg BG. A prevalence survey of Parkinson's disease and other movement disorders in the people's Republic of China. Arch Neurol. 1985:42:655–657.
8. Zhang ZX, Román GC. Worldwide occurrence of Parkinson's disease: An updated review. Neuroepidemiolog. 1993;12(4):195–208.
9. Pan T, Jankovic J, Le W. Potential therapeutic properties of green tea polyphenols in Parkinson's disease. Drugs Aging. 2003;20(10):711–721.
10. Gao X, Cassidy A, Schwarzschild MA, Rimm EB, Ascherio A. Habitual intake of dietary flavonoids and risk of Parkinson disease. Neurology. 2012:78(15):1138–1145.
11. Chan DK, Woo J, Ho SC, Pang CP, Law LK, Ng PW, Hung WT, Kwok T, Hui E, Orr

K, Leung MF, Kay R. Genetic and environmental risk factors for Parkinson's disease in a Chinese population. J Neurol Neurosurg Psychiatry. 1998;65(5):781–784.

12. Checkoway H, Powers K, Smith-Weller T, Franklin GM, Longstreth WT, Swanson PD. Parkinson's disease risks associated with cigarette smoking, alcohol consumption, and caffeine intake. Am J Epidemiol. 2002;155(8):732–738.
13. Tan EK, Tan C, Fook-Chong SMC, Lum SY, Chai A, Chung H, Shen H, Zhao Y, Teoh ML, Yih Y, Pavanni R, Chandran VR, Wong MC. Dose-dependent protective effect of coffee, tea, and smoking in Parkinson's disease: A study in ethnic Chinese. J Neurol Sci. 2003;216(1): 163–167.
14. Ascherio A, Zhang SM, Hernan MA, Kawachi I, Colditz GA, Speizer FE, Willett WC. Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Ann Neurol. 2001;50:56–63.
15. Preux PM, Condet A, Anglade C, Druet-Cabanac M, Debrock C, Macharia W, Couratier P, Boutros-Toni F, Dumas M. Parkinson's disease and environmental factors. Matched caseecontrol study in the limousin region, France. Neuroepidemiology. 2000;19(6):333–337.
16. Henning SM, Wang MP, Abgaryan N, Vicinanza R, de Oliveira DM, Zhang YJ, Lee RP, Carpenter CL, Aronson WJ, Heber D. Mol. Nutr. Food Res. 2013;57: 483–492.
17. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 1983;16: 109–110.
18. Charles B. Breckenridge, Nicholas C. Sturgess et al. Pharmacokinetic, neurochemical, stereological and neuropathological studies on the potential effects of paraquat in substantia nigra pars compacta and striatum of male C57BL/6J mice. Neuro Toxicology. 2013;37:1-14.
19. Drury RAB, Wallington EA. Carleton's histological technique, 5th ed. Oxford, UK: Oxford University Press, Oxford. Wenk modification is substitution of acetaldehyde for paraldehyde. J. Histotechnology. 1996;19(4):353-355.
20. Luna LG. Histopathologic methods and color atlas of special stains and tissue artifacts; Am erican Histolabs, Inc. 1992:483-485.
21. Meppelink AM, de Jong BM, Renken R, Leenders KL, Cornelissen FW, van Laar T. Impaired visual processing preceding image recognition in Parkinson's disease patients with visual hallucinations. Brain. 2009;132:2980–93.
22. Van Eimeren T, Monchi O, Ballanger B, Strafella AP. Dysfunction of the default mode network in Parkinson disease: A functional magnetic resonance imaging study. Arch Neurol. 2009;66:877–83.
23. Onofrj M, Taylor JP, Monaco D, Franciotti R, Anzellotti F, Bonanni L, et al. Visual hallucinations in PD and Lewy body dementias: Old and new hypotheses. Behav Neurol. 2013;27:479–93.
24. Kim JS, Kim JM, OJJ, Jeon BS. Inhibition of inducible nitricoxide synthase expression and cell death by (-)-epigallocatechin-3-gallate, a green tea catechin, in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine mouse model of Parkinson's disease. J. Clin. Neurosci. 2010;17:1165–1168.
25. Levites, Y., Weinreb, O., Maor, G., Youdim MB, Mandel S. Green tea polyphenol (-)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6 tetrahydropyridine-induced dopaminergic neurodegeneration. J. Neurochem. 2001;78:1073–1082.
26. Pan T, Jankovic J, Le W. Potential therapeutic properties of green tea polyphenols in Parkinson's disease. Drugs Aging. 2003;20(10):711–721.
27. Higdon JV, Frei B. Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. Crit Rev Food Sci Nutr. 2003;43(1):89–143.
28. Amit T, Avramovich-Tirosh Y, Youdim MBH, Mandel S. Targeting multiple Alzheimer's disease etiologies with multimodal neuroprotective and neurorestorative iron chelators. FASEB J. 2008;22(5):1296–130.
29. Zhang MH, Luypaert J, Fernandez Pierna JA, Xu QS, Massart DL. Determination of total antioxidant capacity in green tea by near-infrared spectroscopy and multivariate calibration. Talanta. 2004; 62(1):25-35.

30. Munkyong Pae, Zhihong Ren, Mohsen Meydani, Fu Shang, Donald Smith, Simin Nikbin Meydani, Dayong Wu. Dietary supplementation with high dose of epigallocatechin-3-gallate promotes inflammatory response in mice. The Journal of Nutritional Biochemistry. 2012;23(6):526–531.

31. Weisburger JH, Rivenson A, AL CAga C, Reinhardt J, Kellof GJ, Boone CW, et al. Effectof tea extracts, polyphenols, and epigallocatechin gallate on azoxymethane induced colon cancer. Proc Soc Exp Biol Med. 1998;217:104-8.

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