Original Article

Effectiveness of Nigella Sativa soft capsule on tremor in patients with multiple sclerosis: a double-blind clinical trial study

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ABSTRACT

The purpose of the study was to evaluate the effectiveness of Nigella Sativa on tremors in patients with multiple sclerosis. 31 patients were randomly divided into two groups in a double-blind clinical trial study. The Fahn-Tolosa-Marin Tremor Rating Scale and Health Survey were used. Inclusion criteria were tremor due to MS, EDSS score between 2 and 6, having stable and regular drug treatment in the last 3 months, not using alternative drugs from 7 days before the start of the intervention until the end of the intervention, and exclusion criteria were, history of alcohol or substance use disorder, pregnancy or breastfeeding, kidney or liver dysfunction, steroid treatment during one month before the start of the study, neurological comorbidities in addition to MS, heart disease, allergy to Nigella Sativa and an attack of the disease in the last month. Analyses were performed using SPSS-22. The average age of the participants in the case group was 40.12±10.46 and in the control group were 32.26±9.26. It was showed that there is no significant relationship between the demographic data in the two groups (P-value=0.447). The statistical comparison of rest, postural, and tension tremor variables did not show a significant relationship between the two groups. The statistical analysis between the two groups showed that there was no significant relationship between quality of life questionnaires and the clinical tremor rating scale. This study showed that there were no significant differences between the case and control groups in any of the tremor characteristics.

Keywords: Nigella Sativa, Multiple Sclerosis, Tremor, Soft Capsule

Introduction

Multiple sclerosis (MS) is an unpredictable disease characterized by a highly variable disease onset and clinical course [1]. Three main phenotypes have been described, relapsing–remitting MS (RRMS), primary progressive MS (PPMS), and secondary progressive MS (SPMS) [2-4]. Multiple sclerosis symptoms may destroy any function that is fully or partially controlled by the central nervous system [5]. MS has several symptoms such as optic nerve inflammation, Spinal Cord Injury, instability in walking, brain stem syndromes (including vertigo, double vision, facial numbness), and urinary system diseases. MS disease mainly produces a large amplitude of 2.5-7 Hz postural or intention tremor, both of them are classified by the movement disorders under the term action tremor [6-8].

In the last few years, especially after the COVID-19 pandemic, several studies were performed to evaluate the antiviral and anti-inflammation properties of N. sativa. Due to its molecular targets and effects, N. sativa is known to have potential for this purpose. Therapeutic decision-making is more accurate and evidence-based when the results of different clinical trials are published. [9] Currently, the drugs used in the treatment of MS are not completely efficient and patients suffer from their side effects [4, 9, 10]. Today, the trend to use alternative complementary medicines, a suitable lifestyle, and complementary alternative treatment can alleviate some symptoms and improve the quality of life in these patients [4]. Also, the use of natural and herbal products has increased significantly due to fewer side effects and availability [4]. Also, many findings have shown that some plant compounds have improved myelin repair and inhibit inflammation [11, 12]. Nigella sativa has clear effects on the treatment of neurological diseases such as headache, memory impairment, epilepsy, and parkinsonism [13-17]. In addition, it has been reported that Nigella sativa increases the remyelination

of the central nervous system reduces the inflammatory process and suppresses the expression of TGFB1 in experimental autoimmune encephalomyelitis models of MS disease [18]. Folarin et al. found that Nigella sativa has the effects of protecting and restoring nerves and motor coordination in essential tremors [19]. Tremor is one of the relatively common symptoms of MS and generally has a negative impact on the quality of life. Also, due to the side effects of various drugs and the absence of effective treatment for MS, we decided to evaluate the effects of Nigella sativa herbal soft capsule on the tremor of the patients.

Materials and Methods

Study design

This study is a double-blind clinical trial, and the research population includes patients with MS in Khuzestan province.

Inclusion criteria were as follows: tremor based on clinical criteria, EDSS score between 2 and 6, routine treatment in the last 3 months and not participating in another study at the same time.

Exclusion criteria were as follows: a history of alcohol or substance use, pregnancy or lactation, impaired renal or hepatic function, steroid treatment within one month prior to the start of the study, having a neurological disease in addition to MS, heart diseases and there is an allergy to Nigella sativa.

In a double-blind study, 32 patients were randomly divided into two groups. The method of assigning the intervention to the patients was a random and randomized block method. All patients continued their treatment regimen as before, also, the Nigella sativa soft capsule (Pardaneh®) and placebo soft capsule, were prepared in the same packaging so that they do not have any difference in appearance. Sativa has not been used in previous studies in MS patients with tremors, so the dose was determined based on two previous studies: in one study, 12-20 mg/kg of N. sativa oil was administered to inhibit the formation of reactive oxygen species (ROS) in animal models of seizures [20], in another study, 1- 2 grams of N. sativa oil seed extract was used daily for neurotoxicity [21]. The duration of treatment of chronic disease was from 28 days to 3 months.

Data collection

Written informed consent was taken from the patients. At the beginning of the visit (time zero), history and examination were taken and EDSS was determined (SE-36 HEALTH SURVEY questionnaire), then, case group used Nigella sativa 500 mg softgel capsules every 12 hours for 6 weeks and the control group used a placebo soft capsule every 12 hours for 6 weeks. It should be noted that at the end of the first week of taking the drug/placebo, the patients were checked over the phone in terms of clinical conditions and possible side effects. Then, at the end of the 6th week, the patients were asked about their history and possible complications. Also, EDSS was calculated the quality of life questionnaire was completed, and patients were evaluated with the motion analysis system.

The tremor measurement and motion analysis system

The severity of the tremor was recorded in two stages, before and after intervention (placebo). The tremor was measured using a clinical questionnaire and also by recording hand kinematics using an optoelectronic motion capture system (8 Vero 2.2 camera, Vicon Inc. UK). For the clinical measurement of tremor, tremor part of the Fahn-Tolosa-Marin Tremor rating scale questionnaire was used.

To record hand kinematics during rest, postural, and intention tremor experiments, retroreflective markers, 5 mm in diameter, were attached bilaterally on the proximal part of the intermediate phalange of the index finger, proximal to the second and fifth metacarpal (on hamate and trapezium), radius-styloid process, and ulna-styloid process. Amplitude and frequency of hand tremor was measured using the 3D positions of the attached markers which were recorded with 200 Hz frequency.

Tremor specifications for postural and intention tremor test conditions were quantified with measures in the spatial and frequency domain. First, the coordinates of markers were filtered using a bidirectional 9th-order bandpass Butterworth filter with cut-off frequencies of 2 and 25 Hz. Then the mean position of 4 markers on the hand was calculated. The average traveled distance of the hand position and the standard deviation of its acceleration were calculated. The average displacement velocity of the four markers was also calculated.

Short-time Fourier transform was also applied to the resultant spatial signal. The resultant spatial signal was calculated as the Euclidian distance between the hand position and its centroid (as the rooted sum of squares)[22]. Most of the frequency content of the essential tremor lies between 2.5 and 8 hz. Tremor power ratio was calculated as the area under the power spectral density between 2.5-8 Hz divided by the area under the 2-25 Hz [23]. This measure quantifies the essential tremor content of the resultant spatial tremor signal. Also, the average and maximum amplitude in the amplitude spectra of the resultant spatial signal in the range of 2.5-8 Hz was calculated.

For the intention tremor experiments, the smoothed trajectory of the index finger marker was calculated by applying a low pass filter with a 2 Hz cut-off to its position during the reaching duration. Reaching duration was calculated as the time when the velocity of the index finger goes above and again comes back below 10 percent of the maximum of its movement velocity. The Root Mean Squared deviation between the smoothed and actual position was calculated. The average of the standard deviation of the acceleration of the finger marker in 3 directions was calculated as the acceleration dispersion of the finger [24]. Also, the average displacement velocity of the index finger during reaching duration was calculated.

In the frequency domain, the tremor power ratio, and average and maximum amplitude in the amplitude spectra of the finger marker position in each direction, again in the range of 2.5-8 Hz

was calculated. Averaged values of three directions were reported.

All the analysis was performed offline using custom written MATLAB scripts.

Statistical Analysis

Data from the MS patients were analyzed using Analyze of Covariance (ANCOVA) and Independent Samples Test. The results are expressed as mean ± standard deviation (S.D). Statistical analyses were performed using SPSS software (version 26). Values of p≤0.05 were considered statistically significant.

Sample Size

The sample size in this article (n=31) was estimated with an error level of 5% and confidence of 80% through the following relationship.

$$n = \frac{(z_1 - \frac{\propto}{2} + z_1 - \beta)^2 (s_1 + s_2)^2}{d^2} \qquad (1)$$

Results and Discussion

In this study, a total of 31 people were divided into two groups. The mean age of the patients in the case group was 40.12±10.46 and in the control group was 32.26±9.26. The analysis showed that there was no significant relationship between the demographic data in case and control groups (P-value=0.447) (table 1). Data from the MS patients were analyzed using Analyze of Covariance (ANCOVA) and Independent Samples Test. The results are expressed as mean ± standard deviation (S.D).

Table 1. Demographic data of the participants

Variable		Placebo	Case	p-value
		Mean±Sd		
EDSS score		2.50±1.64	1.969±1.20	0.306
Age		9.26±32.26	10.46±40.12	0.447
duration of diagnosis		121±89.9	135.3±117.8	*0.708*
		frequency (percent)		
Gender	female	6(40)	11(68.75)	0.447
	male	9(60)	5(31.25)	
dominant side	Left	5(33.3)	6(37.5)	0. 553
	right	10(66.6)	10(62.5)	
Type of MS	SP	2(12.5)	2(12.5)	
	RR	13(81.25)	14(87.5)	0.658
	PP	1(6.25)	0	

Table 1. The demographic data of the people participating in the study. The average age of the patients in the case group was 40.12±10.46 and in the control group was 32.26±9.26. It was shown that there is no significant relationship between the demographic data in the two groups. (P-value=0.447)

Table 2. The results of statistical tests to determine the effectiveness of Nigella sativa soft gel capsules on Rest tremor in patients with MS (ANCOVA test)

Variable		(placebo Mean±Sd)		(case Mean±Sd)		p-value
		Post	Pre	post	pre	
Amplitude	Standard deviation of hand movement acceleration (millimeters per square second)	221.41±128.83	246.94±140.99	167.922±49.9	324.17±261.61	*0.128*
	Average marker movement speed in space (mm/s)	1.12±2.37	1.06±2.55	1.97±0.56	2.85±1.36	0.148
	Average displacement of the marker in space (mm)	2.03±5.67	2.87±6.50	5.12±0.95	3.5±7.32	0.353
	Power ratio of tremor frequencies to total power (percentage)	7.21±21.38	6.53±20.44	18.79±7.15	3.71±17.08	*0.96*
Frequency	The average power of the frequency spectrum in the tremor range (watts per Hz)	3.54±2.02	22.11±6.81	0.81±0.96	1.77±1.38	0.292
	The maximum power of the frequency spectrum in the tremor range (watts per Hz)	9.33±4.93	37.11±11.79	1.73±1.8	3.04±2.58	0.15

Table 2, shows the statistical results to determine the effect of the results of statistical tests to determine the effectiveness of N. sativa soft capsule on rest tremor in patients with MS in two drug and placebo groups. The findings show that the average standard deviation of hand movement acceleration decreased from 324.17±261.61 to 167.922±49.9 in the drug group, but this decrease is not statistically significant.

Table 3, shows the statistical results to determine the effect of statistical tests to determine the effectiveness of N. sativa soft capsule on postural tremor in patients with MS in two drug and placebo groups. The findings state that the average standard deviation of hand movement acceleration decreased from 307.2±1.219 to 244.26±129.55 in the drug group, but this decrease is not statistically significant.

Table 3. The results of statistical tests to determine the effectiveness of N. sativa soft gel capsules on postural tremor in patients with MS (ANCOVA test)

Variable		placebo Mean±Sd)(		case Mean±Sd)(		p-value
		Post	pre	post	pre	
Amplitude	Standard deviation of hand movement acceleration (millimeters per square second)	272.7±221.63	209.87±324.45	244.26±129.55	307.2±219.1	0.987
	Average marker movement speed in space (mm/s)	11.28±9.71	6.29±8.97	3.98±6.58	4.39±7.51	0.732
	Average displacement of the marker in space (mm)	12.21±19.46	11±20.59	8.15±15.13	2.59±17.59	0.692
Frequency	Power ratio of tremor frequencies to total power (percentage)	5.57±33.46	6.12±32.52	31.22±3.8	4.48±30.69	0.646
	The average power of the frequency spectrum in the tremor range (watts per Hz)	24.08±10.49	39.53±16.02	3.36±4.02	7.56±5.97	0.725
	The maximum power of the frequency spectrum in the tremor range (watts per Hz)	66.18±25.48	120.12±44.78	8.94±8.16	15.8±11.35	0.244

Table 4. The results of statistical tests to determine the effectiveness of N. sativa soft gel capsules on tension tremor in patients with MS

Variable		Placebo		Case		p-value
		Post	pre	post	Pre	
Amplitude	Standard deviation of hand movement acceleration (millimeters per square second)	1135.34±731.66	1407.87±1814.07	1487.75±1194.79	1691.6±659.1	0.244
	Average marker movement speed in space (mm/s)	72.08±62.17	60.2±74.63	64.14±65.18	72.62±81.33	0.352
	The average power of the frequency spectrum in the tremor range (watts per Hz)	274.66±337.41	201.3±287	304.76±339	381.1±528.2	*0.345*
Frequency	The maximum power of the frequency spectrum in the tremor range (watts per Hz)	586.45±654.34	490±579.48	621.29±795.51	802±1262	0.262

Table 4, shows the statistical results to determine the effect of the results of statistical tests to determine the effectiveness of N. sativa soft capsule on Intension tremor in MS patients in two drug and placebo groups. The findings show that the average standard deviation of hand movement acceleration decreased from 1691.6±1.659 to 1487.75±1194.79 in the drug group, but this decrease is not statistically significant.

Table 5. Statistical data of quantitative variables of Short form 36 Health Survey questionnaire (physical & mental component) in two groups

Variable	(sf36ms Mean±Sd)		(sf36ps Mean±Sd)	
groups	Post	Pre	Post	pre
case	52.52±20.5	53±21.85	46.7±22.48	47.88±26.76
Placebo	54.61±19.3	54.15±19.75	48.64±16.8	45.94±16.51
p-value	*0.366*		0.458	

Table 5, shows the statistical results of the analysis of the quantitative parameters of the SF36 questionnaire in two drug and placebo groups. The findings show that the mean and standard deviation of the score in the physical section in the drug group was 47.88±26.76 and after the intervention was 47.88±26.76. In the placebo group, this score was 47.88±26.76 and 47.88±26.76. This difference is not statistically significant. (p -value = 0.566 and test)

Table 6. Statistical information of quantitative variables of clinical tremor rating scale questionnaire in two groups

Variable	(Cts - lft Mean±Sd)		(Cts - rt Mean±Sd)	
groups	Post	Pre	Post	pre
case	1.75±1.84	2±1.93	1.31±1.01	1.37±1.20
Placebo	2.4±2.02	2±1.46	2.86±2.38	2.4±1.24

p-value	*0.358*	*0.137*

Table 6, shows the statistical results of the analysis of the quantitative parameters of the clinical tremor rating scale questionnaire in two drug and placebo groups.

A total of 31 people were divided into two groups 15 control and 16 case and control groups. The aim of the study was to investigate the effect of N .sativa on tremor in patients with MS, characteristics such as amplitude and acceleration, frequency, hand displacement size, etc. The results showed that the N .sativa does not create a significant difference between the two groups in any of the tremor characteristics. Although, based on the results, it has led to a slight improvement in tremor in the case group, but the improvement was not significant compared to the control group. Different studies have been done on the effect of N .sativa in various diseases, but it seems that no study has been done on the effect of this plant on tremors in patients with MS.

Demyelination in autoimmune encephalomyelitis, like MS, is the result of inflammatory lesions in the white matter responsible for clinical deficits. Neuropathology of the active lesion in the CNS of rats with relapsing EAE shows a severe inflammatory response, early demyelination, and axonal loss, in chronic situations, These lesions are gradually depleted of axons. [25-27]. Recently, a laboratory study by Alami et al. [28] showed that extracts of N. sativa have an anti-inflammatory effect on primary mixed glial cells prepared from rat cerebellum. Several studies have shown that N. sativa oil contains potent but non-toxic compounds that suppress excessive inflammatory molecules [29-31].

The majority of action tremors (postural and intention) in patients with MS have been related to the cerebellum as the most likely source of tremor, while the rarity of resting tremors is against the involvement of the basal ganglia. The common occurrence of bilateral tremors may indicate that damage to the cerebellum is often multifocal [32]. Beheshti et al.'s study showed that Nigella sativa, due to its active component (thymoquinone), is widely used to treat CNS diseases such as memory impairment, epilepsy, pain, neurotoxicity, etc. Also, its components can be considered a promising factor in the treatment of CNS disease [17].

In the study of Folarin et al. due to the presence of glutamine peroxidase (GPX) and its antioxidant effect, Nigella sativa reduced phenol-induced essential tremor in rats. Also, Nigella sativa has some effects such as improving motor coordination, regeneration nerve protection, and weight loss [19]. Noor et al. found that Nigella sativa, causes improvement in induced autoimmune encephalomyelitis in rats, by reducing TGFB1 and inflammation and improving remyelination in the cerebellum [18]. Also, in the study of Bamusi et al., they found that Nigella sativa with a dose of 1 mg/day improved the glycemic status and decreased FBG, HBA1C, and 2HGP parameters, but these changes were not significant, although by increasing the dose to 2 mg per day, all parameters improved significantly [33].

The present study seems to be the first study that has been conducted on tremor in patients with multiple sclerosis. This study showed that Nigella sativa has improved the quality of life in terms of mental, physical, and clinical scale tremors in the case group. This improvement can be due to the improvement of hand movement acceleration and the reduction of hand movement displacement, which has led to the reduction of tremors that are in the pathological range of 2.5-8. Also, in a person who has intention tremor, the time of the starting movement from the origin to reaching the destination, and the amount of tremor in the range of motion from near the target to the target has improved, which can also be the effect of the drug. To the best of our knowledge no studies have evaluated the effect of N. sativa on tremor of patients with MS and most of the published studies are limited to the pre-clinical level. There is a need for large scale trials to completely clarify the therapeutic effects of N .Sativa. In our small-sized trial, we cannot deduce that N. Sativa is an effective remedy for MS-related tremors nor enhances the quality of life at 1000 mg/d doses. N .Sativa has a large interval of safety with very low toxicity. The drug group in this study did not show any side effects compared to the placebo group, therefore further studies with higher doses of N. sativa (e.g. 2000 mg/d) might be effective since animal model studies have shown to enhance remyelination in CNS and reduce inflammation models of MS disease [34]. As Ciesielska-Figlon et al. have described in their paper, studies on N. Sativa use in humans are scarce and there might be a difference when the seed extract is used compared to the oil (the latter is used in our study)[35].

Conclusion

This study showed that there was no significant difference between the case and control groups in any of the tremor characteristics.

Limitation Suggestion

Among the limitations of the present study is the limitation in the number of patients. Also, in the initial proposal, the patients were supposed to be analyzed based on the type of MS, but because most of the people were of the RRMS type, it was not possible to do it separately. Also, if the patients are examined on a larger scale, it can be shown that the improvement of the quality of life of the patients will be significant, so one of the limitations of the present study was the small number of participants.

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References

1. Kalincik T., Diouf I., Sharmin S., Malpas C., Spelman T., Horakova D., Havrdova E.K., Trojano M., Izquierdo G., Lugaresi A., et al. Effect of Disease-Modifying Therapy on Disability in Relapsing-Remitting Multiple Sclerosis over 15 Years. Neurology. *2021; 96: e783–e797.*
2. Kappos L, Wolinsky JS, Giovannoni G, et al. Contribution of relapse-independent progression vs relapse-associated worsening to overall confirmed disability accumulation in typical relapsing multiple sclerosis in a pooled analysis of 2 randomized clinical trials. JAMA Neurol. *2020; 77(9): 1132-1140*
3. Pozzilli C, Pugliatti M, Vermersch P, Grigoriadis N, Alkhawajah M, Airas L, Oreja-Guevara C. Diagnosis and treatment of progressive multiple sclerosis: A position paper. European Journal of Neurology. *2023 Jan;30(1):9-21.*
4. Krieger SC, Sumowski J. New insights into multiple sclerosis clinical course from the topographical model and functional reserve. Neurol Clin. *2018;36(1):13-25.*
5. Reipert B. Multiple sclerosis: a short review of the disease and its differences between men and women. Journal of Men's Health and Gender. *2004;1(4): 334-40.*
6. Koch M, Mostert J, Heersema D, De Keyser J. Tremor in multiple sclerosis. Journal of neurology. *2007;254(2):133-45.*
7. Lyons KE, Pahwa R. Deep brain stimulation and tremor. Neurotherapeutics. *2008;5(2):*331-8.
8. Deuschl G, Bain P, Brin M, Committee AHS. Consensus statement of the movement disorder society on tremor. Movement disorders. *1998;13(S3): 2-23.*
9. Elgohary S, Elkhodiry AA, Amin NS, Stein U, El Tayebi HM. Thymoquinone: A Tie-Breaker in SARS-CoV2-Infected Cancer Patients? Cells. *2021 Feb 2;10(2):302.*
10. Rinaldi F, Perini P, Calabrese M, Rinaldi L, Gallo P. Severe relapses after the first infusion of natalizumab in active relapsing—remitting multiple sclerosis. Multiple Sclerosis Journal. *2009;15(11):* 1359-62.
11. Mohajeri M, Sadeghizadeh M, Najafi F, Javan M. Polymerized nano-curcumin attenuates neurological symptoms in EAE model of multiple sclerosis through down regulation of inflammatory and oxidative processes and enhancing neuroprotection and myelin repair. Neuropharmacology. *2015;*99:156-67.
12. Piao Y, Liang X .Chinese medicine in diabetic peripheral neuropathy: experimental research on nerve repair and regeneration. Evidence-Based Complementary and Alternative Medicine. *2012*;*2012.*
13. Fahmy HM, Noor NA, Mohammed FF, Elsayed AA, Radwan NM. Nigella sativa as an anti-inflammatory and promising remyelinating agent in the cortex and hippocampus of experimental autoimmune encephalomyelitis-induced rats. The Journal of Basic & Applied Zoology. *2014;67(5):182-95.*
14. Meles, D.K.; Safitri, E.; Mustofa, I.; Susilowati, S.; Putri, D.K.S.C. Immunomodulatory Activity of Black Jinten Oil (Nigella sativa) as Macrophage Activator for Salmonella typimurium Infected Rat. Indian Vet. J. *2020, 97, 12-14.*
15. Li H, DaSilva NA, Liu W, Xu J, Dombi GW, Dain JA et al. Thymocid, a Standardized Black Cumin(Nigella sativa) Seed Extract, Modulates Collagen Cross-Linking, Collagenase and Elastase Activities, and Melanogenesis in Murine B16F 10 MelanomaCells. Nutrients. *2020;12(7):2146.*
16. Louis ED, Galecki M, Rao AK. Four essential tremor cases with moderately impaired gait: how impaired can gait be in this disease? Tremor and other hyperkinetic movements. *2013;3.*
17. Beheshti F, Khazaei M, Hosseini M. Neuropharmacological effects of Nigella sativa. Avicenna journal of phytomedicine. *2016;6(1):104.*
18. Noor N, Fahmy HM, Mohammed FF, Elsayed AA, Radwan NM. Nigella sativa amliorates inflammation and demyelination in the experimental autoimmune encephalomyelitis-induced Wistar rats. International journal of clinical and experimental pathology. *2015*;*8*(*6*):*6269.*
19. Folarin R, Surajudeen O, Omotosho E, Owoniyi A, Oyeleye D, Shallie P. Motor co-ordinative roles of Nigella sativa oil in mice models of phenol-induced essential tremor. Annals of Health Research. *2020;6(1):85-99.*
20. Javidi S, Razavi BM, Hosseinzadeh H. A review of Neuropharmacology Effects of Nigella sativa and Its Main Component, Thymoquinone. Phytother Res. *2016 Aug;30(8):1219-29.*
21. Hannan MA, Rahman MA, Sohag AA, Uddin MJ, Dash R, Sikder MH, Rahman MS, Timalsina B, Munni YA, Sarker PP, Alam M. Black cumin (Nigella sativa L.): A comprehensive review on phytochemistry, health benefits, molecular pharmacology, and safety. Nutrients. *2021 May 24;13(6):1784.*
22. A Bermeo, M Bravo, M Huerta, A Soto. A system to monitor tremors in patients with Parkinson's disease. Annu Int Conf IEEE Med Biol Soc. *2016:5007-5010.*
23. Mcgurrin P, Mcnames J, Wu T, Hallett M, Haubenberger D. Quantifying Tremor in Essential Tremor Using Inertial Sensors-Validation of an Algorithm. IEEE J Transl Eng Health Med. *2021; 9: 2700110.*

24. Mitsui Y, Thi Zin T, Ishii N, Mochizuki H. Imaging Tremor Quantification for Neurological Disease Diagnosis. Sensors. *2020; 20(22): 6684.*
25. Trapp BD, Bö L, Mörk S, Chang A. Pathogenesis of tissue injury in MS lesions. J Neuroimmunol. *1999; 98: 49-56.*
26. Brown A, McFarlin DE, Raine CS. Chronologic neuropathology of relapsing experimental allergic encephalomyelitis in the mouse. Lab Invest. *1982; 46: 171-85.*
27. Cross AH, McCarron R, McFarlin DE, Raine CS. Adoptively transferred acute and chronic relapsing autoimmune encephalomyelitis in the PL/J mouse and observations on altered pathology by intercurrent virus infection. Lab Invest. *1987; 57: 499-512.*
28. Alemi M, Sabouni F, Sanjarian F, Haghbeen K, Ansari S. Anti-inflammatory effect of seeds and callus of Nigella sativa L. extracts on mix glial cells with regard to their thymoquinone content. AAPS Pharm Sci Tech. *2013; 14: 160-7.*
29. Padhye S, Banerjee S, Ahmad A, Mohammad R, Sarkar FH. From here to eternity - the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond. Cancer Ther *2008; 6: 495-510.*
30. Shahzad M, Yang X, Raza Asim MB, Sun Q, Han Y, Zhang F, Cao Y, Lu S. Black seed oil ameliorates allergic airway inflammation by inhibiting T-cell proliferation in rats. Pulm Pharmacol Ther *2009; 22: 37-43.*
31. Butt MS, Sultan MT. Nigella sativa: reduces the risk of various maladies. Crit Rev Food Sci Nutr 2010; 50: 654-65.
32. Deuschl G, Raethjen J, Lindemann M, Krack P (2001) The pathophysiology of tremor. Muscle Nerve *24:716–735*
33. Bamosa AO, Kaatabi H, Lebdaa FM, Elq AM, Al-Sultanb A. Effect of Nigella sativa seeds on the glycemic control of patients with type 2diabetes mellitus. Indian J Physiol Pharmacol. *2010;54(4):344-54.*
34. Dalli M, Bekkouch O, Azizi SE, Azghar A, Gseyra N, Kim B. *Nigella sativa* L. Phytochemistry and Pharmacological Activities: A Review (2019-2021). Biomolecules. *2021 Dec 23;12(1):20.*
35. Mihoubi W, Sahli E, Rezgui F, Dabebi N, Sayehi R, Hassairi H, Masmoudi-Fourati N, Walha K, Ben Khadhra K, Baklouti M, Ghzaiel I, Fattouch S, Menif H, Mokdad-Gargouri R, Lizard G, Gargouri A. Whole and Purified Aqueous Extracts of Nigella sativa L. Seeds Attenuate Apoptosis and the Overproduction of Reactive Oxygen Species Triggered by p53 Over-Expression in the Yeast Saccharomyces cerevisiae. Cells. *2022 Mar 3;11(5):869.*