In Silico Prediction of Potential Compounds of Nigella sativa as Aromatase Agonists and Ability to Cross the Blood-Brain Barrier for treatment of Alzheimer's Disease

Kusuma Andriana[1,2], Nurdiana[3], Wisnu Barlianto[4,7], I Wayan Arsana Wiayasa[5,7], Masruroh Rahayu[6,7]

[1]Lecturer, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Muhammadiyah Malang, [2]Student, Doctoral Program of Medical Science, Faculty of Medicine, Universitas Brawijaya, [3]Professor, Department of Pharmacology, Faculty of Medicine, Universitas Brawijaya, [4]Lecturer, Department of Pediatric, Faculty of Medicine, Universitas Brawijaya, [5]Lecturer, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Brawijaya, [6]Lecturer, Department of Neurology, Faculty of Medicine, Universitas Brawijaya , [7]Physician, Saiful Anwar General Hospital, Malang, East Java, Indonesia

Abstract

Background: Alzheimer's disease (AD), is a chronic neurodegenerative disease which condition is characterized by a decline in memory, thinking skills, and the ability to perform basic activities of daily living. Study showed that postmenopausal women with AD had a lower aromatase activity (p450 aromatase) in the brain compared to menopausal women without AD. To improve cognitive function in AD patients, Nigella sativa (NS) was found to have a protective effect on memory, and cognitive function. This study therefore, aimed to investigate in silico prediction of potential compounds of Nigella sativa as aromatase agonists and ability to cross the Blood-Brain Barrier (BBB) for treatment of Alzheimer's Disease. **Methods:** The data sets used in this study were collected from databases KnapSack Kanaya, PubChem, and PASS server Way2Drug. HitPick and Autodock Vina was performed. Additionally, the systematic analysis involved ADMET, LMMD, and SWISS ADME. **Results and Discussions:** In silico test results revealed the most promising constituents in 36 active NS compounds that may have potential to reduce the severity of the AD, owing to its anti-inflammatory, cytochrome p450 stimulants, free radical scavengers, antioxidants, and immunomodulators. The ability of NS to cross the BBB was proven by admetSAR LMMD with an analysis average value of 0.91 (from the highest value of 1). Further, NS can trigger cytochrome P450 aromatase activity via Quercetin 3-(6'' ''-feruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside which has a better binding affinity value than its control (androstenedione). NS through oleic acid compounds may bind to peroxisome proliferator-activated receptor gamma (PPRAG), similar to Rosiglitazone which may affect transcription and activation regulation of PPARG. **Conclusion:** To be concluded, Nigella sativa could be used as a potential medicinal plant for the treatment of Alzheimer's disease.

Keywords: *In silico, Nigella sativa, Aromatase agonists, Blood-Brain Barrier, Alzheimer's Disease*

Corresponding author:
Kusuma Andriana
Lecturer, Department of Obstetrics and Gynecology, Faculty of Medicine,
University of Muhammadiyah Malang
Jl. Bendungan Sutami 188A, Malang, East Java, Indonesia, Email: andriana.k.umm@gmail.com

Introduction

Alzheimer's disease (AD), is a chronic neurodegenerative disease which condition is characterized by a decline in memory, thinking skills, and the ability to perform basic activities of daily living. AD had been also identified as the most common cause of dementia in later life[1-3]. The increased risk of

developing severe symptoms of AD was also associated with many factors including brain estrogen deficiency in females with AD. A previous study showed that postmenopausal women with AD had a lower aromatase activity (p450 aromatase) in the brain compared to menopausal women without AD[4].

Many studies have been searching for ways to cure AD, but none of the drugs can stop the progression of disease[5]. Currently, the treatment strategy of AD may reduce some physical symptoms and controlling behavioral symptoms that impact daily life. Interestingly, a study found that 25% of the treatment regimens today were related to natural products. The increased use of herbal products is due to their affordable and accessible choice, fewer side effects in contrast to synthetic drugs, and a more effective selection in patients with chronic diseases[6]. The usage of natural products increases when prescribed medicines were ineffective in the treatment of chronic diseases. The identification of potential drugs to effectively inhibit the progression of AD is crucial, thus many natural plants including *Nigella sativa* (NS) are also being proposed for its phytopharmaceuticals in the treatment of AD[7-9].

With respect to improve cognitive function in AD patients, Nigella sativa (NS) was found to have a protective effect on memory, and cognitive function. NS has a powerful constituent of antioxidants and protects against damage induced by the oxidative processes and the abilities to binding to protein targets. Thus, it may prevent neuronal cell death and improve cognitive function[8,10-12]. NS, as a herbal medicine has been widely recognized for its potential biological activities, such as a diuretic, antihypertensive, antidiabetic, anticancer, immunomodulatory, antimicrobial, anthelmintic, analgesic, a spasmolytic, bronchodilator, anti-inflammatory, antitussive, gastroprotective, hepatoprotective, reduce LDL, renal protective and antioxidants[13-20]. In addition, the essential oil of NS seeds contains various active ingredients, such as thymoquinone (TQ) (30–48%), thymol, thymohydroquinone (THQ), dithymoquinone, p-cymene (7–15%), carvacrol (6–12%), sesquiterpene longifolene (1 –8%), 4-terpineol (2–7%), t-anethol (1–4%), and α – pinene[7], nigellimine-N- oxide, nigellicine, nigellidine and alpha-hederin[21]. Further, the antioxidant and anti-inflammatory activities of NS play a crucial role to help prevent oxidative damage in cells, especially in the brain area[10].

Due to its natural plants, a safety, and efficient along with favorable phytopharmaceuticals properties, NS has been suggested to be a potential for further study in the treatment of AD. This study therefore, aimed to investigate in silico prediction of potential compounds of Nigella sativa as aromatase agonists and ability to cross the Blood-Brain Barrier (BBB) for treatment of Alzheimer's Disease.

Materials and Methods

The data sets used in this study were collected from databases KnapSack Kanaya and PubChem. KnapSack Kanaya revealed the potential biological activities of NS compounds. Meanwhile, the PubChem database was used for identifying the SMILE (simplified molecular-input line-entry system) structure of these compounds. The SMILE structure was employed for tracing and exploration of NS compounds using the PASS server Way2Drug. Basically, the PASS server Way2Drug identified the probability activator (Pa), and probability inhibitor (Pi). Pa value describes the potential properties of NS for AD treatment, while the Pi value represents the potential inhibitors of the NS compounds. Pa value > 0.7 means that the input compounds have a high efficacy of similarity with the compounds that have been proven in the database as AD treatment. However, if the predictive value between 0.3-0.7, then the compound will computationally be known to play a vital role in the treatment of AD, yet the similarity to compounds that have been proven in certain activities, could be less.

HitPick was used for predicting NS compounds-protein target interactions. The higher the value of prediction precision (maximum 100%), and similarity (highest is 1), the higher the probability of these compounds can interact. After that, String-db was used to identify multi protein interaction. In addition, to evaluate the binding affinities/docking scores between ligands (NS compounds) and Aromatase (PDB ID 3EQM), Autodock Vina was performed, using PyRx 9.5 program. Docking results was evaluated based on the binding affinity value. The more negative the value, the stronger the interaction between ligands and protein. PyMol 2.3 program 1 was used to visualize docking results, while the LigPlot 2.1 program was used

to see amino acid interactions. The docking grid box was as follows: Center X: 87,914 Y: 54,097 Z: 44,738 Dimension Armstrong: X: 4,542 Y: 15,916 Z: 32,648.

Additionally, the systematic analysis involved Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET), Laboratory of Molecular Modeling and Design (LMMD), and SWISS ADME. These are tools for predicting and analyzing drug pharmacokinetics. The higher the value, the better the ability to penetrate, and the highest value of this analysis is 1[22].

Results and Discussion

In silico test results revealed the most promising constituents in 36 active NS compounds that may have potential to reduce the severity of the AD, owing to its anti-inflammatory, cytochrome p450 stimulants, free radical scavengers, antioxidants, and immunomodulators. Tabel 1 showed the results of database tracing using KnapSack, there were 36 active compounds in NS. Further exploration was carried out using the Pass server Way2 Drug to determine the potential of NS compounds as AD therapy.

Table 1: Probability active components in *Nigella sativa* (NS)

Metabolites	Anti-inflammation	Cytochromes p450 stimulants	Cytochromes p450 inhibitor	Free radical scavengers	Antioxidants	Immunostimulants
Thymol	0.564	0.396		0.348	0.314	
Carvacrol	0.673	0.396		0.295	0.313	0.225
alpha-Thujene	0.807	0.35				
alpha-Pinene	0.476	0.442		0.160		
beta-Pinene	0.601	0.308				
Myrcene	0.287	0.325		0.38	0.469	0.622
Lauric acid	0.515	0.305		0.315	0.229	0.504
Oleic acid	0.614	0.273		0.36	0.283	0.549
Anisaldehyde		0.755	0.273	0.35	0.233	0.284
Apiol	0.469	0.174		0.459	0.358	0.167
Estragole	0.447	0.389	0.259	0.486	0.337	0.205
Myristicin	0.38		0.19	0.552	0.359	0.206
(+)-R-Citronellol	0.53	0.26		0.365	0.588	0.585
p-Cymene	0.643	0.517	0.213	0.365	0.144	0.173
(+)-Fenchone		0.369				0.184
alpha-Phellandrene	0.395	0.447				
gamma-Terpinene	0.463	0.441		0.163		0.237
Longifolene	0.57	0.256		0.157	0.208	0.225
(Z.Z.Z)-Octadeca-9.12.15-trienoic acid	0.812	0.255		0.281	0.363	0.504
Thymoquinone	0.6	0.48		0.238	0.229	0.242
Kaempferol 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	0.751			0.977	0.926	0.677
Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	0.753			0.997	0.928	0.639

Metabolites	Anti-inflammation	Cytochromes p450 stimulants	Cytochromes p450 inhibitor	Free radical scavengers	Antioxidants	Immunostimulants
Quercetin 3-(6’’’’-feruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside	0.737			0.997	0.889	0.482
Salfredin B11	0.519	0.357		0.485	0.422	
Fuzitine						
Nigellicine	0.426			0.174		
Nigellidine	0.698	0.307		0.218	0.159	
Nigellimine	0.318	0.395	0.278	0.219		
4-Terpineol	0.603	0.312		0	0.151	
4(10)-Thujene	0.853	0.25	0.173	0		
Nigeglanine	0.48	0.218		0		
Nonane	0.424	0.517		0.266	0.170	0.372
Carvone	0.469	0.322		0.15	0.193	
alpha-Longipinene	0.377	0.3		0.18	0.164	
Dihydrocarvone	0.476	0.35			0.172	
Nigellidine 4-O-sulfite					0.165	
Mean	0.554	0.360	0.231	0.321	0.351	0.373
SD	0.147	0.115	0.041	0.265	0.235	0.179

This study found that the highest mean of probability active components in NS, Pa value of anti-inflammatory activities = 0.554. There were several compounds that had Pa > 0.7, which were investigated as potential compounds based on in silico and laboratory tests, known as anisaldehyde (Pa = 0.755) for Cytochrome p450 Stimulant. Anti-inflammatory activity was found in 6 compounds with the highest Pa of 0.853 in compound 4(10)-Thujene. Free radical scavengers and anti-oxidant activities were found in 3 compounds, namely Kaempferol 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside Quercetin 3-(6’’ ‘’-feruloylglucosyl)-(1->2)-galactosyl-(1->2) -glucoside and Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside. The highest Pa of 0.997 for free radical scavenger activity was found in Quercetin 3-(6’’ ‘’-feruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside and Quercetin 3-glucosyl-(1->2)-galactosyl-(1-> 2)-glucoside. The highest Pa of 0.928 for anti-oxidants was found in Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside.

Hitpick analysis was performed to determine the target protein of the active NS compounds. The input compounds included in the Hitpick program were 36 active NS compounds. After data processing, 10 protein targets were obtained, namely Fatty Acid Binding Protein 7 (FABP7), FABP4, FABP3, Peripheral myelin protein 2 (PMP2), Ganglionic Long-Term Potentiation (GLTP), Free fatty acid receptor1 (FFAR1), FABP5, FABP1, GC and albumin (ALB). These target proteins were protein targeted by oleic acid. The selection of protein targets was based on similarity and target precision, namely 1.0 and 100%, indicating that the target based on the HitPick database has a high confidence score. Several proteins act as biomarkers in Alzheimer’s such as: FABP7, FABP4, FABP3. Other protein targets were associated with PMP2, GLTP, FABP5 and FABP1 neurogenesis and neurogenesis. Meanwhile, other proteins were considered as proteins that were widely found in the body such as ALB, so they were not specific.

In addition, using the String-db program, protein interaction analysis determined the interactions of the target protein. From the Hitpick results, eight proteins were entered into the String-db program and were then analyzed. The result obtained was FABP5 interaction

which activated VEGFA protein which was known as a neuroprotective cytokine that induced neurogenesis and angiogenesis. VEGFA protein had a positive effect on reducing Alzheimer's symptoms, as well as depressive symptoms in adults, increasing VEGF protein in the hippocampal region and neurogenesis[23]. The String-db data also showed that VEGFA activated FABP4, which was one of the biomarkers in frontotemporal dementia[24].

Furthermore, the analysis of the interaction of oleic acid with peroxisome proliferator-activated receptor gamma (PPARG) was a ligand that activates transcription factors with their biological activities regulating glucose metabolism, fat and suppression of inflammatory gene expression[25]. Oleic acid was predicted to have interactions with PPARG. The interactions of compounds and proteins were predicted using the STITCH-DB program.

This study showed that oleic acid could bind to PPARG and the interaction that occurred was transcriptional regulation. Administration of PPRAG agonists had been shown to improve AD pathology in AD experimental animals. Likewise, the administration of Rosiglitazone - a PPARG agonist - demonstrated a significant improvement in memory and cognition in AD patients. Rosiglitazone was previously given to treat type 2 diabetes mellitus (DM) as well as thiazolidinedione (TZD) which was also a PPARG agonist[25-26]. Rosiglitazone could influence transcription and activation regulation of PPARG. From the results of the compound and protein interaction analysis, it was possible that NS could be used as a potential medicinal plant for AD drugs with the PPARG target protein.

The aromatase enzyme was an important enzyme in the process of steroidogenesis, a family of cytochrome P450, and was encoded by the CYP19 gene on chromosome 15[27-28]. P450arom, also known as estrogen synthase, was a terminal enzyme complex from the steroidogenesis pathway that catalyzes the conversion of C19 androgens (androstenedione and testosterone) to C18 estrogens (estrone and E2). This enzyme complex consists of cytochrome P450 aromatase and flavoprotein NADPH-cytochrome. Aromatase was involved in various brain functions such as synaptic plasticity, neurogenesis, neuroprotection and regulation of sexual and emotional behavior in mice, was also involved in the pathophysiology of AD and autism spectrum disorder (ASD) in humans[29]. To determine the role of the active NS compound in triggering the activity of cytochrome P450 aromatase, an affinity bond comparison was carried out with the control 4-ANDROSTENE-3-17-DIONE which had bound to aromatase. Of the 37 docking ligands, there was one ligand that had a better value than the control, namely Quercetin 3-(6'' ''-feruloylglucosyl) - (1->2) -galactosyl-(1->2)-glucoside.

Table 2: admetSAR analysis using Laboratory of Molecular Modeling and Design (LMMD) to predict Nigella sativa (NS) permeability to cross the blood-brain barrier (BBB)

Metabolites	BBB Penetration Score	
Thymol	0.9381	BBB+
Carvacrol	0.9381	BBB+
alpha-Thujene	0.9550	BBB+
alpha-Pinene	0.8959	BBB+
beta-Pinene	0.9229	BBB+
Myrcene	0.9478	BBB+
Lauric acid	0.9488	BBB+
Oleic acid	0.9539	BBB+
Anisaldehyde	0.9073	BBB+
Apiol	0.9399	BBB+
Estragol	0.9383	BBB+

Cont... **Table 2: admetSAR analysis using Laboratory of Molecular Modeling and Design (LMMD) to predict Nigella sativa (NS) permeability to cross the blood-brain barrier (BBB)**

Metabolites	BBB Penetration Score	
Myristicin	0.9504	BBB+
(+)-R-Citronellol	0.9409	BBB+
p-Cymene	0.9677	BBB+
(+)-Fenchone	0.9849	BBB+
alpha-Phellandrene	0.9049	BBB+
gamma-Terpinene	0.9431	BBB+
Longifolene	0.9687	BBB+
(Z,Z,Z)-Octadeca-9,12,15-trienoic acid	0.9314	BBB+
Thymoquinone	0.8138	BBB+
Kaempferol 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	0.9144	BBB+
Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	0.7248	BBB-
Quercetin 3-(6’’’’-feruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside	0.9251	BBB+
Salfredin B11	0.8952	BBB+
Fuzitine	0.7793	BBB-
Nigellicine	0.6906	BBB-
Nigellidine	0.8590	BBB+
Nigellimine	0.9868	BBB+
4-Terpineol	0.9104	BBB+
4(10)-Thujene	0.9554	BBB+
Nigeglanine	0.9890	BBB+
Nonane	0.9821	BBB+
Carvone	0.8689	BBB+
alpha-Longipinene	0.9400	BBB+
Dihydrocarvone	0.9256	BBB+
Nigellidine 4-O-sulfite	0.7214	BBB-

The blood–brain barrier (BBB) was a term used to describe the unique properties of the microvascular central nervous system (CNS), which was a semipermeable, highly selective boundary and separated circulating blood from the brain from deep extracellular fluid (CNS)[30]. This system allowed the passage of water, some gases, and fat-soluble molecules by passive diffusion, as well as the selective transport of molecules such as glucose and amino acids which are essential for nerve function. BBB LMMD was an analysis to predict whether these compounds could penetrate the BBB. Additionally, 36 secondary metabolites of NS were analyzed using the SAR LMMD admet with the highest value of the analysis being 1. The higher the value (close to 1), the better its ability to penetrate BBB[31]. From this analysis in Table 2, it was found 10 metabolites with an analysis number > 0.95 and 3 metabolites that were unable to cross the BBB, namely Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside, Fuzitine, and Nigellidine 4-O-sulfite. The average value of the analysis was 0.91. However,

the ability of NS and TQ to cross the BBB still needs to be studied further[8]. Thymoquinone and THQ in the form of glycosidic, binding to aglycone which then allowed it to cross the BBB[32]. Thymoquinones could cross the BBB and had neuromodulator activities[33].

Conclusion and Acknowledgement

This study showed 36 active NS compounds, and the most potential properties were anti-inflammatory. Other biological activities of NS compounds were Cytochrome p450 Stimulants, free radical scavengers, anti-oxidants, and immunomodulators. Further, NS had an ability to cross the BBB and there was one ligand of Quercetin 3-(6'' '-eruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside which showed a better value than its control (androstenedione). Quercetin could trigger cytochrome P450 aromatase activity. Oleic acid could bind to PRAG similar to Rosiglitazone which then affected transcription regulation and activation of PPARG. In conclusion, Nigella sativa could be used as a potential medicinal plant for the treatment of Alzheimer's disease. This study would not have been possible without the support of Doctoral Program of Medical Science, Faculty of Medicine, Universitas Brawijaya and Department of Obstetrics and Gynecology, Faculty of Medicine, University of Muhammadiyah Malang. I am especially grateful to all of those with whom I have had the pleasure to work during the study.

Conflict of Interest: The authors declare that there is no conflict of interest.

Source of Funding: This study was financially supported by Faculty of Medicine, University of Muhammadiyah Malang.

Ethical Clearance: Ethical approval for this study was obtained from the research ethics committee of Universitas Brawijaya, No. 043-KEP-UB-2020.

References

1. Kumar A, Singh A, Ekavali. A review on Alzheimer's disease pathophysiology and its management: an update. Pharmacological Reports 2015;67(2):195–203.
2. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. Alzheimers Dement (N Y) 2018;4:575-590.
3. Mizuno S, Iijima R, Ogishima S, Kikuchi M, Matsuoka Y, Ghosh S, Miyamoto T, Miyashita A, Kuwano R, Tanaka H. AlzPathway: a comprehensive map of signaling pathways of Alzheimer's disease. BMC Syst Biol. 2012;6:52.
4. Yue X, Lu M, Lancaster T, Cao P, Honda S, Staufenbiel M, et al. Brain estrogen deficiency accelerates Abeta plaque formation in an Alzheimer's disease animal model. Proc Natl Acad Sci U S A. 2005;102(52):19198-203.
5. Kolata G. An Alzheimer's Treatment Fails: 'We Don't Have Anything Now' [Internet]. New York Times online; 2020 Feb 10 [cited 2020 Dec 10]. Available from: https://www.nytimes.com/2020/02/10/health/alzheimers-amyloid-drug.html.
6. Wachtel-Galor S, Benzie IFF. Herbal Medicine: An Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2011. Chapter 1. Available from: https://www.ncbi.nlm.nih.gov/books/NBK92773/.
7. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed. 2013;3(5):337-52.
8. Cascella M, Bimonte S, Barbieri A, Del Vecchio V, Muzio MR, Vitale A, et al. Dissecting the Potential Roles of Nigella sativa and Its Constituent Thymoquinone on the Prevention and on the Progression of Alzheimer's Disease. Front Aging Neurosci. 2018;10:16.
9. Sallehuddin N, Nordin A, Bt Hj Idrus R, Fauzi MB. Nigella sativa and Its Active Compound, Thymoquinone, Accelerate Wound Healing in an In Vivo Animal Model: A Comprehensive Review. International Journal of Environmental Research and Public Health 2020;17(11):4160.
10. Sahak MK, Kabir N, Abbas G, Draman S, Hashim NH, Hasan Adli DS. The Role of Nigella sativa and Its Active Constituents in Learning and Memory. Evid Based Complement Alternat Med. 2016;2016:6075679.
11. Andriana K, Yuliartha DL. Effect of Extract Black Cumin (Nigella sativa) on the Vaginal Cytology of

Ovariectomized White Mouse (Rattus Novergicus Strain Wistar). In: Pratiwi ID, Kurnia AD; Rofida S, editors. Proceedings of the Health Science International Conference (HSIC 2017); Malang. The Netherlands: Atlantis Press; 2017. p. 357-361. Available from: https://www.atlantis-press.com/proceedings/hsic-17/25885871.

12. Safithri F, Andriana K. Black Cummin Extract (Nigella sativa Linn) on Spatial Memory Performance in Menopausal Model of Rat. In: Pratiwi ID, Kurnia AD; Rofida S, editors. Proceedings of the Health Science International Conference (HSIC 2017); Malang. The Netherlands: Atlantis Press; 2017. p. 254-263. Available from: https://www.atlantis-press.com/proceedings/hsic-17/25885856.
13. Abel-Salam BKA. Immunomodulatory effects of black seeds and garlic on alloxan-induced Diabetes in albino rat. Allergol Immunopathol (Madr). 2012;40(6):336-40.
14. Keyhanmanesh R, Gholamnezhad Z, Boskabady MH. The relaxant effect of Nigella sativa on smooth muscles, its possible mechanisms and clinical applications. Iran J Basic Med Sci. 2014;17(12):939-49.
15. Keyhanmanesh R, Nazemiyeh H, Mazouchian H, Bagheri Asl MM, Karimi Shoar M, Alipour MR, et al. Nigella sativa Pretreatment in Guinea Pigs Exposed to Cigarette Smoke Modulates In Vitro Tracheal Responsiveness. Iran Red Crescent Med J. 2014;16(7):e10421.
16. Abdel-Daim MM, Ghazy EW. Effects of Nigella sativa oil and ascorbic acid against oxytetracycline-induced hepato-renal toxicity in rabbits. Iran J Basic Med Sci. 2015;18(3):221-7.
17. Gholamnezhad Z, Boskabady MH, Hosseini M. Effect of Nigella sativa on immune response in treadmill exercised rat. BMC Complement Altern Med. 2014;14:437.
18. Pourbakhsh H, Taghiabadi E, Abnous K, Hariri AT, Hosseini SM, Hosseinzadeh H. Effect of Nigella sativa fixed oil on ethanol toxicity in rats. Iran J Basic Med Sci. 2014;17(12):1020-31.
19. Mousavi G, Mohajeri D. Effect of ground black seeds (Nigella sativa L.) on renal tubular cell apoptosis induced by ischemia/reperfusion injury in the rats. Iran J Basic Med Sci. 2014;17(12):1032-5.
20. Durmuşkahya C, Öztürk M. Ethnobotanical Survey of Medicinal Plants Used for the Treatment of Diabetes in Manisa, Turkey. The Open Conference Proceedings Journal 2013;4:288.
21. Akram Khan M, Afzal M. Chemical composition of Nigella sativa Linn: Part 2 Recent advances. Inflammopharmacology 2016;24:67-79.
22. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7:42717.
23. Echeverria V, Barreto GE, Avila-Rodriguezc M, Tarasov VV, Aliev G. Is VEGF a Key Target of Cotinine and Other Potential Therapies Against Alzheimer Disease? Curr Alzheimer Res. 2017;14(11):1155-1163.
24. Teunissen CE, Elias N, Koel-Simmelink MJA, Durieux-Lu S, Malekzadeh A, Pham TV, et al. Novel diagnostic cerebrospinal fluid biomarkers for pathologic subtypes of frontotemporal dementia identified by proteomics. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 2016;2:86–94.
25. Landreth G, Jiang Q, Mandrekar S, Heneka M. PPARγ agonists as therapeutics for the treatment of Alzheimer's disease. Neurotherapeutics 2008;5(3):481–489.
26. Heneka MT, Reyes-Irisarri E, Hüll M, Kummer MP. Impact and Therapeutic Potential of PPARs in Alzheimer's Disease. Curr Neuropharmacol. 2011;9(4):643-50.
27. Arevalo M-A, Azcoitia I, Garcia-Segura LM. The neuroprotective actions of oestradiol and oestrogen receptors. Nature Reviews Neuroscience 2014;16(1):17–29.
28. Cui J, Shen Y, Li R. Estrogen synthesis and signaling pathways during aging: from periphery to brain. Trends Mol Med. 2013;19(3):197-209.
29. Takahashi K, Hosoya T, Onoe K, Takashima T, Tanaka M, Ishii A, et al. Association between aromatase in human brains and personality traits. Scientific Reports 2018;8(1);16841.
30. Daneman R, Prat A. The blood-brain barrier. Cold Spring Harb Perspect Biol. 2015;7(1):a020412.
31. Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, et al. admetSAR: A Comprehensive Source and Free Tool for Assessment of Chemical ADMET Properties. Journal of Chemical Information and

Modeling 2012;52(11):3099–3105.

32. Jukic M, Politeo O, Maksimovic M, Milos M, Milos M. In Vitroacetylcholinesterase inhibitory properties of thymol, carvacrol and their derivatives thymoquinone and thymohydroquinone. Phytotherapy Research 2007;21(3):259–261.
33. Kassab RB, El-Hennamy RE. The role of thymoquinone as a potent antioxidant in ameliorating the neurotoxic effect of sodium arsenate in female rat. Egyptian Journal of Basic and Applied Sciences 2017;4(3):160–167.