



Neuroprotective effects of *Vitex agnus castus* extract in rats' model of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is the most common degenerative neurological disorders and is characterized by a variety of symptoms such as memory loss, reduced of cognitive functions, abnormal behavior and psychiatric problems. *Vitex agnus castus* (Vac) has been reported to have both antioxidant and neuroprotective effects. The preset study was designed to study the effect of Vac extract in lipopolysacchride - induced AD - like features in rats. Material and method : Rats were divided equally (7 rats/group) into five groups; control group, induction group (rats were administered lipopolysacchride 250 µg/kg i.p. once daily for 7 days), and the further three treatment groups included rats received tested drugs prophylactically for 21 days then the induction done by lipopolysacchride (250 µg/kg) i.p. once daily together with continuation of the same oral doses of the tested drugs for further 7days; these treatment groups included donepezil group (0.5 mg/Kg orally once daily), VCA extract group (80mg/Kg orally once daily) and combination group (donepezil 0.5mg/Kg and Vac extract 80mg/Kg; both were administrated orally once daily). Behavioral parameters like Ymaze and novel object recognition were evaluated. Oxidative stress parameters and inflammatory cytokines were carried out in the sample of brain tissues. Result: vitex agnus castus extract have significant improvement in memory and cognition, and improve the level of oxidative stress parameter, and inflammatory cytokines

Conclusion: The present study revealed the neuroprotective effect of Vac against lipopolysacchride-induced AD -like features in rats. Such beneficial effect seemed probably due to Vac significant antioxidant and anti-inflammatory effects as been proved in the present study.

Keywords: Alzheimer's disease, lipopolysaccharide, *Vitex agnus castus*

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INTRODUCTION

Alzheimer's disease (AD) is a progressive disease usually starts slowly and worsens gradually (over time, more parts of brain will be affected, and more symptoms will be developed) (Alzheimer's association, 2017). The features' keys of AD pathogenesis are a gradual accumulation of a protein fragments beta amyloid outside the neurons and twisted fibers of tau protein inside the neuron (DeTure et al. 2019). Current AD therapy consists of cholinesterase inhibitors (donepezil, rivastigmine and galantamine) and N - methyl - d - aspartate (NMDA) antagonists, including memantine (Haroon et al. 2020). *Vitex agnus castus* (Vac) belongs to the Verbenaceae family with phytoestrogenic properties (Honari et al. 2012) also called monk's pepper or chaste tree which could be found in Mediterranean region, Central Asia, and Southern Europe. Pharmacological effects of Vac include amelioration of premenstrual syndrome symptoms (headache, mood

changes and irritability), improvement of the menopausal symptoms (urogenital, vasomotor, mood dysfunction, and uterine bleeding) (Chopin.2003), and cyclic mastalgia (Dinç and Coşkun 2014). *Vitex agnus castus* may have beneficial effects of on learning and memory, possibly by an increase in ER α gene expression in the hippocampal formation (Allahtavakoli et al. 2016). Because Vac has estrogen-like effects and anti-inflammatory properties; its extraction reduces infarct volume and decreases neurological deficits caused by stroke, which is similar to estrogen effects (Alimohammadi et al.2015).

the study aims To investigate and evaluate the possible neuroprotective effects of Vac extract against lipopolysacchride-induced AD -like features in rats

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MATERIALS AND METHODS

The dried fruit of Vac was washed with distilled water and let dry then grounded to powder then weighted to (500 gm). The Vac powder was extracted by weighting 50g of the powder and placed in a flask, prepare 10 flask then add in each of the ten flasks with 200ml of 100% ethanol with continuous shaking by using the water bath for eight hours at 40°C, then filtration done by using filter paper whatman 18cm. Finally, ethanol was evaporated with a rotary machine and the residual was dried to form a powder. The resultant extract was then reconstituted by solving it in saline before being orally administered to animals (Ankit et al., 2012).

Thirty five male rats with age range of 3-4 months and weight range of 300-500 g were housed under standard laboratory conditions with a temperature of 20-22 °C. The following drugs were used: Lipopolysacchride (LPS) (Sigma-Aldrich, USA), ethanol (Chem.-lab, Belgium), Vac (cultivated in Baghdad) and donepezil (Pfizer) are dissolved normal saline. Ymaze and open field box are locally made in Baghdad.

The animals were divided equally into 5 groups of 7 rats each: Group 1: Negative control group. Group 2 (induction group): rats were received LPS i.p. 250 µg/kg once daily for 7 days to induce AD- like features Zhu et al. 2014). The further three treatment groups included rats received tested drugs prophylactically for 21 days then the induction done by LPS 250 µg/kg i.p. once daily together with continuation of the same oral doses of the tested drugs for further 7days; these treatment groups included group 3 (Donepz group): donepezil 0.5 mg/Kg orally once daily (Qiliang Zhou et al. 2016). group 4 (Vac group): Vac extract 80 mg/Kg orally once daily (Honari et al. 2012), and group 5 (combination group)(Donepz+ VAC): donepezil 0.5mg/Kg and Vac extract 80 mg/Kg; both were administrated orally once daily. Behavioral test including cognitive examination using Y maze and novel object recognition (NOR) were done on day 29 for three successive days.

Behavioral tests

A. Y-Maze Test

The Y-maze is a three-arm horizontal maze with an angle of 120 degrees, which were of 50 cm length, 10 cm width, and 20 cm height. The maze floor and walls were made from white polyvinyl plastic. The three arms are named as A,B,and C respectively. Rats were initially placed in one arm, and then the sequence and number of arm entries were monitored for an 8-min period. An actual alternation was defined when a rat entered into all three arms on consecutive choices (i.e., ABC, BCA, or CAB, but not CAC, BAB, or ABA). The spontaneous alternation (%) was derived from the total number of alternations divided by the total number of arm entries minus two, which was multiplied by 100 as shown in the following equation: % Alternation = [(Number of alternations)/(Total number of arm entries - 2)] × 100.

Table 1. Phytochemical screening of *Vitex agnus castus* extract

Plant part	Alkaloids	Flavonoids	Steroids	Tannins	Saponins	Terpenoids
Extract	+	+	+	+	+	+

The number of arm entries also served as an indicator for movement and locomotor activity (Gil-Yong Lee et al 2017).

B. Novel Object Recognition (NOR)

For the object recognition task, an open field box(40 _ 40 _ 20 cm) composed of black plastic material was utilized as the experimental apparatus..Two similar objects are place in the box. Both objects types presented during the test session varied in texture, color, and size. This assessment has three phases: (i) habituation; (ii) training, and (iii) test. On the first day, each rat was allowed to become familiarized with the open field box without the presence of an object for about 15 min. On the second day, each rat was placed in the open field for 5 min and allowed to freely explore the two similar objects. After an interval of 90 min post-training session, one of the old objects used was substituted with a new object and the rats were subjected to a 2 min test run. The time spent with each object was recorded The recognition index was calculated using the formula $[TB/(TA + TB)*100]$ where TA and TB are time spent exploring familiar object A and novel object B respectively (Batool et al., 2016). Exploration of an object was noted when a rat sniffed or touched the object with its nose and/or forepaws.

After the behavioral tests, animals were anesthetized by inhaled diethyl ether. The animals were sacrificed, and their brains were immediately removed and washed in cold phosphate -buffered saline. The right hemisphere was rinsed in ice-cold PBS (0.02mol/L, pH 7.2-7.4)(Bhattacharyya et al, 2003). The homogenized tissue of right hemisphere was used to assess oxidative stress indices (levels of MDA and SOD₁) and inflammatory cytokines (levels of IL-1β,IL-6, and TNF-α) in rat brain tissues with aid of ELISA (Elabscience) according to the manufacturer's recommendations accordingly.

Most of data were presented as mean± standard deviation. Unpaired *t*-test was used for comparison of means of two groups, and ANOVA (analysis of variance) for means of three groups or more. Statistical package for social sciences (SPSS) version 23 were used to analyze the results. P values < 0.05 or ≤0.001 were considered significant or highly significant successively (Goodman,1993).

RESULTS

The Phytochemical screening of *Vitex agnus castus* extract show positive results for all compound (**Table 1**).

Y-maze test : The spontaneous alteration highly significantly decreased ($p \leq 0.001$) in induction group when being compared to control group, while each of

Table 2. Effects of *Vitex agnus castus* extract and donepezil on behavioral tests

Group	Y Maze	NOR
Control	85.53±17.47	82.07±9.27
Induction (LPS)	43.0±8.5 [#]	43.36±11.4 ^{##}
Donepez	83.6±11.37 ^{**}	81.56±5.53 ^{**}
Vac	81.19±14.26 ^{**}	73.02±9.87 ^{**}
Combination (Donepez+Vac)	84.15±5.3 ^{**}	79.63±10.77 ^{**}

Donepez: Donepezil, Vac: *Vitex agnus castus*, LPS: Lipopolysacchride, NOR: novel object recognition, n = 7 rats/group, Data are expressed as mean ± SD,

Statistically significant (p≤0.05), ## Highly statistically significant (p≤0.001) compared with control group,

*: Statistically significant (p< 0.05), **: Highly statistical significant (p≤0.001) compared with induction (LPS) group.

Table 3. Effects of *Vitex agnus castus* extract and donepezil on oxidative stress parameters

Group	MDA (ng/ml)	SOD ₁ (ng/ml)
Control	118.81±4.01	0.97±0.13
Induction(LPS)	273.41±20.9 ^{##}	0.73±0.05 ^{##}
Donepez	121.85±2.63 ^{**}	0.85±0.02 ^{**}
Vac	122.17±3.03 ^{**}	0.86±0.02 [*]
Combination (Donepez+Vac)	121.8±3.75 ^{**}	0.89±0.04 [*]

Donepez: Donepezil, Vac: *Vitex agnus castus*, LPS: Lipopolysacchride, n = 7 rats/group, Data are expressed as mean ± SD,

Statistically significant (p< 0.05), ## Highly statistically significant (p≤0.001) compared with control group,

*: Statistically significant, **: Highly statistical significant compared with induction (LPS) group.

Donepez, Vac and combination (Donepez + Vac) groups showed highly statistically significant increase in the spontaneous alteration in comparison to induction group and there was no statistically change (p>0.05) when compared with control group (**Table 2**).

NOR test: The recognition index highly significantly decreased in induction group when being compared to control group. While each of Donepez, Vac and combination (Donepez + Vac) groups showed highly statistically significant increase in comparison to induction group and there was no statistically change when compared with control group (**Table 2**).

Assessment of oxidative stress

Compared to control group, induction group showed highly significant increase in MDA level and highly significant decrease in SOD₁. Each of Donepez, Vac and combination (Donepez+Vac) groups showed highly significant decrease in MDA levels and significant increase in SOD₁ levels compared to induction group. Compared to MDA and SOD₁ level of control group, both Vac and combination (Donepez + Vac) groups showed statistically non-significant change (p>0.05). Regarding comparison with SOD₁ level in control group, Donepez group showed a significant decrease while MDA levels showed statistically non-significant change (**Table 3**).

Assessment of inflammatory cytokines

Compared to control group, there was a highly significant increase in IL-1 β , IL-6 and TNF α levels in induction group. While in comparison to induction group, each of Donepez, Vac and combination (Donepez + Vac) groups showed highly significant decrease in IL-1 β , IL-6 and TNF α levels. There was non-significant changes in IL-1 β , IL-6 and TNF α levels in Donepez, Vac and

Table 4. Effects of *Vitex agnus castus* extract and donepezil on inflammatory cytokines

Group	IL-1 β (pg/ml)	IL-6 (pg/ml)	TNF- α (pg/ml)
Control	75.39±21.83	51.96±8.5	119.8±8.9
Induction (LPS)	198.95±47.91 ^{##}	155.72±44.13 ^{##}	598.98±203.78 ^{##}
Donepez	81.72±16.14 ^{**}	59.09±7.92 ^{**}	123.57±6.52 ^{**}
Vac	89.74±16.52 ^{**}	61.63±8.4 ^{**}	125.55±4.77 ^{**}
Combination (Donepez+Vac)	80.01±21.07 ^{**}	54.82±7.63 ^{**}	122.63±8.15 ^{**}

Donepez: Donepezil, Vac: *Vitex agnus castus*, LPS: Lipopolysacchride, n = 7 rats/group, Data are expressed as mean ± SD,

Statistically significant (p< 0.05), ## Highly statistically significant (p≤0.001) compared with control group,

*: Statistically significant, **: Highly statistical significant compared with induction (LPS) group

combination (Donepez+Vac) groups when being compared to control group (**Table 4**).

DISCUSSION

Alzheimer's disease is the most common degenerative neurological disorders. It is multi-factorial in nature with an unknown etiology that is characterized by several pathological manifestations, including abnormal accumulation of beta-amyloid (A β) and microtubule tau protein, death of cholinergic neurons, abnormal activity of acetylcholinesterase (AChE), dyshomeostasis of metals such as copper, iron and zinc, and metal-induced oxidative stress (Morrone et al. 2019; Yu-Ling et al. 2019). In this study, cold method or maceration method for extraction was used to avoid any loss or destruction of the compounds inside the fruit from exposure to high temperature (Hayder et al., 2014). Phytochemical analysis of Vac extract found phytoconstituent which included flavonoids, terpenoids and glycosides; when comparing our results of phytochemical analysis was comparable with many studies (Abu-Raghif et al. 2015).

In the present study, LPS was used to induce neurotoxicity rat model that mimics AD. It is frequently used to induce an animal model of neuro-inflammation (Salmani et al. (2018) Studies had been proven that LPS injection is able to induce cognitive impairment in mice as it induces memory loss and amyloidogenesis *in vivo* and *in vitro* (Jeong et al. 2017). LPS acts on TLR4/CD14/TLR2 receptors of leukocyte and microglia, the harmful effects of LPS due to the release of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α and proinflammatory mediators such as inducible NOS (iNOS), PGE2, and COX2 from macrophages and other type of cells (Jeong et al. 2017). Peripheral systemic administration of LPS has shown to produce neuro-inflammation and oxidative stress in the brain (Ho et al., 2015). which increase A β level (Zhan et al. 2018). By conducting NOR and Y-maze tests, the present study confirm that LPS interferes with learning and memory functions and subsequently causes impairment of learning and short-term as well as long-term memories. Besides, Increased levels of MDA and reduced level of the activity of SOD₁ and inflammatory cytokines in brain

tissue of the LPS group relative to the control group have confirmed an oxidative stress and inflammatory status that follow LPS administration.

Natural products could be a source of neuroprotective drugs because of they can maintain normal cellular interaction in the brain and decrease the loss of neuronal functions in pathological conditions as in the progression of AD, and associated beneficial health effects and few side effects (Butterfield and Boyd-Kimball.2018).Conventional wisdom in traditional medicine suggests that, due to the synergy of its components, the whole part of a herb, is more effective than its single compositions (Foster, 2012).

Results of the present study showed that Vac extract could achieve significant improvement in cognitive functions, oxidative stress parameters, and inflammatory cytokines levels to be comparable to those of control (apparently healthy) group.

Vitex agnus castus extract was reported to improve learning and memory in the ovariectomized rats that comparable with estradiol, this effect of Vac extract may be associated with an increase in ER α mRNA in the hippocampal formation (Allahtavakoli et al.2016). *Vitex agnus castus* has neuroprotective effects and can reduce stroke injuries comparable to estrogen by estrogenic and anti-inflammatory effects (Alimohammadi et al.2015).Antioxidant and anti-inflammatory properties may be involved in beneficial

effects of Vac on learning and memory (Moreno et al., 2015).

Vitex agnus castus reduces oxidative stress because it contains complex mixture of flavonoids, tannins and phenolic compounds, so these compounds have an important role in the scavenging of the free radicals and therefore they are considered to occur naturally as a source of potential antioxidant (Abu-Raghif et al. 2015).

Vitex agnus castus has reported to increase the SOD and catalase activities and to reduce the MDA level in D-galactose aging mice (Ahangarpour et al. 2016). Ibrahim et al. showed that the administration of Vac protects the lung from oxidative stress induced by LPS that causes pulmonary injury.

Vitex agnus castus has anti-inflammatory effects by a significant decrease in the release of pro-inflammatory cytokines such as TNF- α and IL-6 from macrophages, which are mediated by many critical events for the initiation of acute, subacute and chronic inflammation. Also, it has an anti-proliferative activity and scavenging the free radicals (Chhabra and Kulkarni 2014).

CONCLUSION

Result of this study demonstrated that Vac extract improves learning and memory in LPS induced AD-like features possibly by a mechanism associated anti-inflammatory and antioxidant effects.

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