Original Article

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The Effects of the Ethanolic Extract of Vitex Agnus Castus on Stroke Outcomes in Ovariectomized Mice

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ABSTRACT

BACKGROUND AND OBJECTIVE: The prevalence of stroke in premenopausal women is lower than men. In fact, estrogen is known as a potent neuroprotective agent in cerebral ischemia before menopause. Phytoestrogens are considered to be less risky than chemical estrogens. In this study, considering the phytoestrogenic properties of Vitex agnus custus, the effects of the ethanolic extract of this plant on stroke outcomes in a model of middle cerebral artery occlusion were investigated in ovariectomized mice.

METHODS: In this experimental study, 32 mice, weighing 25-35 g, were randomly divided into 4 groups (8 mice per group): 1) sham group, 2) control group (ovariectomized, treated with 1 ml/kg saline for one month, followed by stroke induction), 3) Vitex group (ovariectomized, treated with 80 mg/kg of Vitex extracts in 1 mL saline every day for a month), and 4) estrogen group (ovariectomized, treated with 40 µg/kg of estradiol valerate in 1 mL saline every day for a month). After one month, stroke was induced in ovariectomized mice by cauterizing the middle cerebral artery. Infarct volume and neurological disorders were evaluated one week after stroke induction.

FINDINGS: A week after stroke induction, infarct volume in the control, estrogen, and Vitex groups was 11.98±2.33, 3.41±1.01, and 5±1.10, respectively. Vitex extracts and estrogen could decrease infarct volume, compared to the control group ($p<0.05$). Also, estrogen and Vitex extracts reduced neurological deficits, compared to the control group ($p<0.001$). A week after stroke induction, sensorimotor disorders in the control, estrogen, and Vitex groups were 50±7, 15±2, and 21±4.09, respectively. In fact, a significant difference was observed between the control and other groups ($p<0.001$).

CONCLUSION: The findings of the present study showed that Vitex extracts, similar to estrogen, have neuroprotective effects, following middle cerebral artery occlusion in ovariectomized mice.

KEY WORDS: Vitex, Ovariectomized, Model of Middle Cerebral Artery Occlusion, Extract.

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Introduction

Stroke is the third leading cause of death and the first cause of disability in adults, worldwide (1). Stroke is defined as the blockage of blood flow in large vessels in the brainthat can cause permanent neurological damages. If not managed promptly;itcanresult in severe functional impairments and even death (2). Stroke occurs in humans as a result of factors such as diabetes, hypertension, aging, cardiac diseases (such as atrial fibrillation), and continuous use of drugs. In addition to the mentioned factors, differences in gender (female and male) may have significant impacts on the mechanism of stroke and response to treatment (3).

The risk of stroke in women before menopause is lower than men of the same age (4). However, the incidence of cerebrovascular events rapidly increases in women after menopause (5).

Studies have shown that estrogen reductiondue to various factors such as surgical interventions (ovariectomy), pharmacological agents (estrogen receptor antagonists) andaging eliminates the protective effect of estrogen in women worsens stroke outcome(6, 7). In addition, estrogen has been identified as a neuroprotective agent in the brain in the past two decades (8).

Although hormone replacement therapy has been extensively applied for postmenopausal women, long-term use of estrogen in these women increases the risk of endometriosis and breast cancer (9). Therefore, we must seek a solution to reduce the side-effects of estrogen in women after menopause as they benefitfrom its protective effects. One of the proposed strategies is the use of herbal estrogens, also known as phytoestrogens. Non-steroidal phytoestrogens are in fact herbal estrogens withestrogen-like structure and function.

This compound is composed of two phenolphthaleins, which bond with estrogen receptors under internal and external conditions (10, 11). As a previous study indicated,soy extract as a phytoestrogen plays a neuroprotective role, following reperfusion after cerebral ischemia (12). Moreover, it has been reported that phytoestrogens reduce infarct volume in a rat model of transient focal stroke (13). Vitex agnus custus belongs tothe Verbenaceae family with phytoestrogenic properties (14). This plant is native to Europe, although it can grow in many parts of the world, particularly in South America (15).

This plant, also called monk's pepper, grows in Iran, as well and is used as a medicinal herb. The constituents of this plant include an alkaloid known as viticin, isoflavones (mainly casticin), and other flavonoids such aspenduletin and crizofanol. The ripe dried fruit of Vitex contains iridoid glycosides and aucubin, containing flavonoids and other essential oils (15). There are also different chemical compounds in the extracts of this plant such as apigenin and penduletin, which mostly affectestrogen receptor beta (16), and linoleic acid, which impacts bothestrogen receptoralpha and beta (17).

Since stroke has serious consequences in postmenopausal women and is more frequent in women than men of the same age and the use of estrogen hormones has irreparable consequences for women, search for a suitable alternative for estrogenic hormones seems essential andphytoestrogens appear to be a good alternative.

Therefore, in this study, the effects of the ethanolic extracts of Vitexagnuscustuson stroke outcomes were investigated in ovariectomized mice.

Methods

This cross-sectional study was conducted in the maternity ward of Ayatollah Rohani Hospital affiliated with the Babol University of Medical Sciences which is a principal referral center for women with high-risk and

premature pregnancies. The sample size was determined on the basis of previous studies. In the present study, 377 pregnant women with premature delivery (23-37 weeks of pregnancy) and 423 sample term pregnant women were enrolled from 2011 to 2013. Upon obtaining permission from the Ethics Committee of the Medical University of Babol, written consent was provided from the subjects.

Through interviewing the mothers, the initial maternal data were extracted including the age, gravidity and parity, any history of abortion, BMI, mother's degree of education and occupation, any history of infertility, smoking habits and the use of alcohol and drugs, consumption of fast food (more than twice a week), the age of pregnancy, cervical cerclage, cell phone use, any history of diseases in the mother (e.g anemia, cardiovascular diseases, diabetes, hypertension and thyroid and psychiatric disorders) (11, 12), any history of surgery, Urinary Tract Infections (UTI), Oligohydramnios, intrauterine growth restriction (IUGR), embryonic anomalies, premature rupture of membranes and vaginal bleeding. Eventually, the infants' data were recorded by a neonatal specialist including birth weight, need for resuscitation and hospitalization, 5-minute Apgar scores and the mortality rate of the hospitalized neonates. The collected data were analyzed and compared by SPSS software V.18, T-test, Chi-square, Fisher's exact test and Mann-Whitney. The $p<0.05$ was considered as significant.

Results

The effect of Vitexextracts on infarctvolume was analyzed a week after stroke induction. The average infarct volume in the control and Vitexgroupswas 11.98±2.33 and 5±1.10, respectively. A significant decrease was observed in infarct volume in the Vitex group, comparedto the control group. In other words, Vitex extracts decreased the infarct volume one week after stroke induction, compared to the control group ($p<0.05$) (fig 1).

Also, estrogen significantly reduced the infarct volume a week after stroke induction, compared to the control group.

The mean infarct volume in the estrogen groupwas 1.01±3.41 a week after stroke, which showed a significant decrease ($p<0.01$) (fig 1). In total, estrogen and Vitex etractsdecreased infarct volume by 80% and 60%, respectively a week after stroke induction. However, no significant differences were observed between estrogen and Vitex groups.

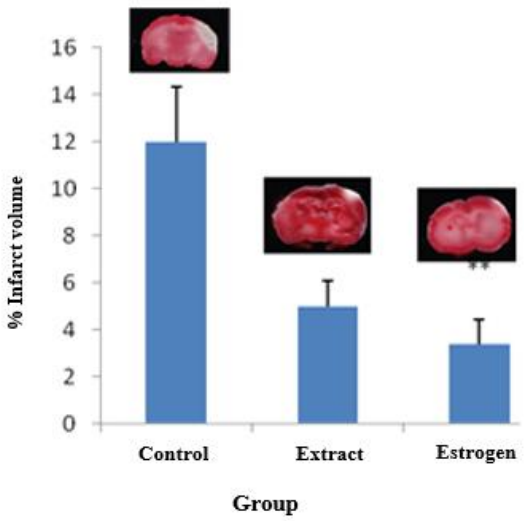

Figure 1. Effects of vitex agnus castus extract and estrogen on infarct volume after stroke induction

*A significant difference compared to control group ($p<0.05$)

**A significant difference compared to the control group($p<0.01$)

Timetotouchand remove the tape from the contralateralpalm was less in the Vitex group, compared to the control group 24 h after stroke induction ($p<0.05$). The corresponding values in the control and Vitexgroupswere 49±13 and 17±3.024 within 24 hours after stroke induction, respectively.

Forty-eight hours after stroke induction, time to touch and remove the tapein the Vitex group was less

than that observed in the control group (p<0.01). The corresponding values were 52±12 and 22±3 in the control and Vitexgroups, respectively. A week after stroke induction, time to touchand remove the tape in the Vitexgroup was less than the control group (p<0.001). These values in the control and Vitexgroups a week after stroke inductionwere 50±7 and 21±4.09, respectively (fig 2).

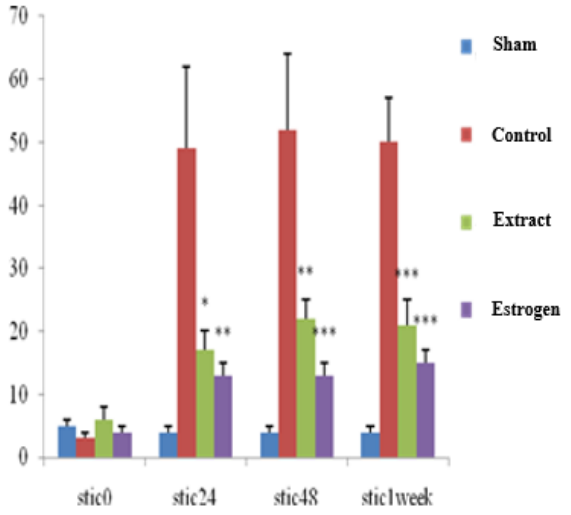

Figure 2. The effects of Vitex Agnus Castus extract and estrogen on sensorimotor disorders At 24 h, 48 h, and one week after stroke

*Compared to the control group (p<0.05)

**Compared to the control group (p<0.01)

***Compared to the control group (p<0.001) (N=8)

Twenty-four hours after stroke induction, time to touchand remove the tape in the estrogengroup was less than the control group (p<0.01). The corresponding values in the control and estrogen groups were 49±13 and 13±2, respectively. Moreover, 48 h after stroke induction, time to touchand remove the tape in the estrogengroup was less than the control group (p<0.001). The corresponding values in the control and estrogen groups were 52±12 and 13±2, respectively.A week after stroke induction, time to touchand remove the tape in the estrogen group was less than the control group (p<0.001). The corresponding values in the control and estrogen groups were 50±7 and 15±2, respectively (fig 2).

Moreover, the effects of Vitex on neurological deficits were studied. Vitex extracts significantly decreased neurological deficits 48 hours (p<0.05) and a week after stroke induction (p<0.01), compared to the control group. Also, estrogen significantly reduced neurological deficitswithin 48 h (p<0.05) and a week after the stroke (p<0.001), compared to the controls (table 1).

Table 1. The effects of Vitexextracts and estrogen on neurological deficits, based on Bederson's ranking system

Groups	Sham	Control	Vitexe	Estrogen
24h	0(0-0)	2(1.25-2)	1(1-2)	1(1-75.1)
48h	0(0-0)	2(2-2)	1(1-2)*	1(1-75.1)
1 w	0(0-0)	2(2-25.4)	1(1-2) **	1(1-1) ***

Data are expressed asmedian and 25th and 75th percentiles (in parentheses).

*Compared to the control group (p<0.05)

**Compared to the control group (p<0.01)

***Compared to the control group (p<0.001) (N=8).

Discussion

The results showed that Vitex significantly reduced infarctvolume, neurological deficits, and motor dysfunction. In a study by Yang and colleagues, it was shown that estrogen has protective effects on brain cells (25). Moreover, Fiocchetti and colleagues reported neuroprotective effects of estrogen (26). Moreover, Hurn et al. demonstrated the protective effects of estrogen on cerebral ischemia (27). The results of our study showed that estrogen reduced infarct volume and neurological disorders resulting from stroke; these results were consistent with the results of previously mentioned studies.

Huang et al. have reportedthat a diet containing isoflavonesimprovesinfarct volume and neurological deficits after transient focal cerebral ischemia in mice. Also, they found that soy isoflavones can maintain the replicativecapacity of neural stem cells and thus reduceinfarct volume and functional impairments after stroke (28). Moreover, Schreihofer and colleagues showed that only two weeks of a high-soy diet in animals resulted in lower infarct volume, compared to animals with a low-soy diet in a focal cerebral infarction model (29).

Lovekamp-Swan and colleagues also suggested that dietary soy reduces infarct volume after MCAO in ovariectomized rats (30). According to theconducted studies, Vitexhas medicinal advantagesand phytoestrogenic effects. As Wuttke et al. indicated, apigenin (a flavonoid) is a compound with agonistic activities for estrogen receptors (16). The flavonoids of this plant demonstrate phytoestrogenic effects (14). Liu and colleagues showed that linoleic acid in Vitexextract is able to bondwith both estrogen receptor alpha and beta; moreover, it possesses effects similar to estrogen (17). A study by Choudharyet al. showed that this extract has anti-inflammatory effects, as well. In addition,the active ingredients of Vitexwere isolated and their anti-inflammatory effects were examined in cell-based contemporary test.It was found that p-hydroxybenzoic acid, 3, 4-hydroxybenzoic acid, and 3, 4-DIhydroxybenzoic have significant anti-inflammatory activities (31). In conclusion, Our study results also showed that Vitex, similar to estrogen, can reduce infarct volume in a permanent stroke model and subsequently decrease neurological and sensorimotor dysfunctions, occurring after stroke. Due to estrogen-like effects andanti-inflammatory properties of Vitex, the extract of this plant reduces infarct volume and thereby decreases neurological deficits caused by stroke, which is comparable with the effects of estrogen. Herbal medications may have some side-effects. In addition, replacement therapies with phytoestrogenic plants may have adverse outcomes in the long term therapy. Therefore, further studies are required to assess the side-effects of this plant. It is suggested that histological changes should be evaluated in target tissuesof estrogen such as uterine and breast to use the obtained results in clinical settings.

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