Effects of *Vitex agnus - castus* Extract on After- Discharge Threshold, Seizure Stages and Kindling Acquisition in Male Rats

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Abstract

Background: ***Vitex agnus - castus*** **extract (Vitex) is available in dosage forms for female disorders treatment. This extract has shown controversial effects against seizures induced by Maximal electroshock (MES) or pentylenetetrazole (PTZ).**

Objective: In the present study the anti-seizure activity of Vitex against acquisition of amygdala kindling was evaluated in male rats.

Methods: Intact male rats were stereotaxically implanted with a tripolar and 2 monopolar electrodes in amygdala and dura respectively. The threshold of AD emerging was determined in each animal. Then, Vitex or solvent was injected and AD threshold was determined again. Also, Vitex injection was continued daily and seizure stages (S1 to S5) and ADDs were recorded 30 min post Vitex injection till development of full kindling.

Results: Vitex treatment increased the AD threshold significantly more than 2.5 times and decreased the after-discharge duration (ADD). Although, the number of trials increased significantly by Vitex for exhibition of stages 1 (S1) to S3, but this effect was not significant for development of S4 and S5 (generalized seizures). The cumulative ADDs difference between control and Vitex group was only significant for S3 - S5.

Conclusions: Vitex may induce a protective effect via increment of stimulation threshold and decrement of ADD at least against focal epilepsy in amygdala neurons. Regarding to its limited effects on kindling acquisition at late stage with generalized seizures, Vitex may postpone the progress of epileptic activity at initial stages.

Keywords: Vitex, Male rats, Amygdala, Kindling acquisition, Seizure

Introduction

The epileptic disorders, especially in refractory forms can prevent the patient's performance and active presence in the society [1]. In many cases, even multi-drug therapy is not effective and in these states patients have to undergo neurosurgical procedures [2]. Since many of herbal drugs have few adverse effects and are produced simply with low-priced technology, the assessment of herbal medications with known few adverse effects, for treatment of epilepsy is worthwhile.

Chaste tree is a deciduous shrub and native to Mediterranean Europe and central Asia. This plant had been completely known in Iranian ancient medical schools as Vitagnus (Vitex) and Alkandi used the plant for treatment of epilepsy and psychosis in 1200 A.D. He mixed the Vitex extract with other extracts and named it Black remedy [3]. Traditionally, Vitex fruit extract has been widely used in the treatment of many female disorders, such as menstrual irregularity, premenstrual syndrome (PMS) and cyclic mastalgia. [4-8]. In vitro investigations has elucidated that the lipophilic extract of Vitex acts as agonist for mu and kappa opioid [8, 9] and also D2 receptors [9, 10], while aqueous fraction has more tendency to bind to delta opioid receptors. The aqueous fraction of methanolic extract inhibits the release of acetylcholine in a concentration-dependent manner [9]. This extract has shown estrogenic effects which may be related to the presence of linoleic acid as an estrogenic compound [7, 11].

In experimental model, the methanolic extract of Vitex negundo leaves has shown significant protection against strychnine and PTZ induced convulsion in mice. [12]. Also, the ethanolic extract of leaves have decreased the number and duration of convulsion in PTZ model, but have not been effective against maximal MES [13].

The electrical kindling model is regarded as one of the excellent experimental model which is very similar to human complex partial seizures. [14, 15]. The antiepileptic activity of different doses of Vitex was demonstrated on kindled seizures parameters in male rats [16].

Based on these investigations and our previous report, this study was designed for more evaluation of the antiepileptic activity of the Vitex extract in the male rats. In the present study the effect of Vitex on AD threshold (before kindling), seizure stages during the kindling and kindling acquisition was evaluated.

Materials and Methods

Animals

Intact and healthy adult male Albino rats weighting 250 - 350 grams, were housed in constant regulated temperature (21±2 °C) 12 h light-dark cycle (lights on 7:00-19:00) and relative humidity (55 ± 20%) with a commercial diet, tap water available and libitum. Animals were acclimated to the laboratory conditions for at least 5 days before surgery.

Extract

Hydroalcoholic extract of Vitex fruit was purchased from Sina Daru Company (Iran).

Surgical and kindling procedures

The animals under the ketamine (50 mg/kg) anesthesia, were stereotaxically implanted with bipolar stimulating and monopolar recording electrodes (twisted into a tripolar configuration) terminating in the basolateral amygdala nucleus of the right

hemisphere (coordinates: *A*, 2.5 mm; *L*, 4.8 and 7.5 mm below dura). Electrodes (stainless steel, teflon coated, A-M Systems, USA) were insulated except at the tips. Two other electrodes were connected to skull screws, placed above the left cortical surface, as ground and reference (differential). Ten days after the surgery, electrical kindling was begun by determining the AD threshold. Kindling stimulation (2 Sec, 100 Hz, monophasic square wave pulses of 0.5 ms per half wave) were initially delivered at 10 µA with an ascending steps by 10 µA at 5 min intervals, until at least 5 seconds of AD was recorded, as previously described [17]. Convulsive stages were identified according to paradigm of Racine (1972): stage 0, no response or motor arrest; stage 1, facial or jaw movements; stage 2, addition of head nodding; stage 3, unilateral forelimb clonus; stage 4, rearing with bilateral forelimb clonus; and stage 5, rearing, forelimbs clonus and loss of equilibrium [18]. Animals were considered to be fully kindled after 5 consecutive stage 5 convulsions for 5 consecutive days.

Drug treatment

Based on our previous study on dose response of Vitex [16] the dose of 60 mg/kg was chosen as suitable dose for this investigation.

After 10 days of recovery from the surgery, the animals were randomly allocated to one of the different groups (n=6-8 per group). The AD threshold was determined in each animal as pretreatment control. Then, on the following days the Vitex extract (60 mg/kg i.p.) or hydroalcoholic solvent (0.1 ml, i.p.) was injected and the AD threshold was determined again 30 minutes post injection. This protocol was continued daily until the animal to reach a constant AD threshold.

To evaluate the Vitex effects on kindling acquisition, the animals stimulated by the first AD threshold or higher to emerge at least 5 sec ADD, daily 30 minutes post either Vitex (60 mg/kg, i.p.) or solvent injection (0.1 ml, i.p.) and the ADD and seizure stages (SS) were recorded in each animal. This procedure was repeated once daily, until full kindling development. Convulsive stage and number of trials to reach each stage were identified. The AD threshold in each animal was determined again at the end of procedure.

Histology

All animals were euthanized by anesthesia at the end of procedure. Their brains were removed, sectioned and examined under microscope for electrode tip placement verification.

Statistical analysis

The results are expressed as mean ± S.E.M. and statistical significance was evaluated by one way ANOVA. $p<0.05$ was taken as significant.

Data expressed as percent of the first stimulation (AD) threshold, were compared within and between groups by Wilcox one and Mann-Whitney U-test respectively. Number of trials or stimulations to full kindle seizures and within each stage was compared using Mann-Whitney U-test.

Result

Anatomical and microscopic observation, verified electrode tips placement in the basolateral amygdale.

The effects of Vitex on AD threshold in intact male rats

To evaluate the effect of Vitex on AD

threshold, intact male rats received either hydro alcoholic solvent or Vitex (60 mg/kg, i.p.) and the AD threshold was determined in each animal 30 min treatment. As shown in Fig. 1, injection of 60 mg/kg of Vitex extract increased significantly the AD threshold when compared to its control group ($p<0.05$). Also, Vitex extract treatment was associated with ADD reduction ($p<0.05$) especially in two initial days, in comparison to the control group (Fig. 2). This effect was reduced at third day of treatment.

The effects of Vitex on kindling acquisition

In this set of experiments the animals received daily either solvent or Vitex (60 mg/kg, i.p.) daily till to reach full kindling. Vitex treatment was associated with a significantly ($p<0.05$) higher numbers of trials to reach stages 1, 2 and 3 (S1-S3) of seizure stages (Fig. 3). Although, the trials to reach full kindling was more than control in Vitex treated group, but the difference was not significant for S4 and S5 of kindling acquisition. The results also showed that chronic Vitex treatment could reduce significantly the cumulative ADDs for S3 to S5 ($p<0.001$), but had no effect on S1 and S2, when compared to the control group (Fig. 4).

The effects of Vitex on the Stability of kindling and seizure stages

While the animals in the control group which received daily hydroalcoholic solvent and reached to the full kindling showed S5 state after a 2 week free from the stimulation, the animals treated with Vitex and reached to full kindling, remitted to S3 or S4 after a two-week interval free of stimulation. The AD threshold at the end of kindling procedure (in Vitex treated group) was not reduced and remained significantly higher than control (Fig. 5).

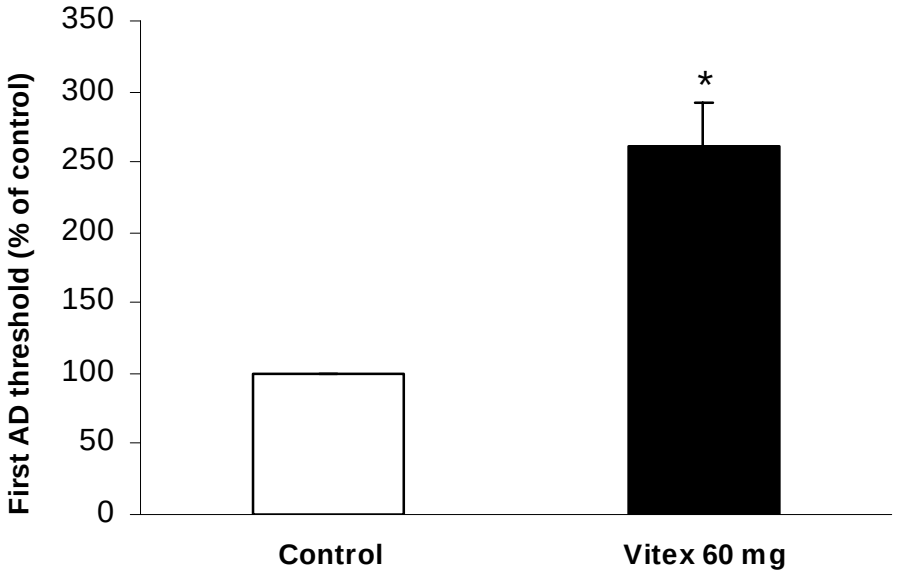

Fig. 1- Effect of *Vitex agnus - castus* on AD threshold in electrical amygdala kindling model of epilepsy in intact male rats. The AD threshold was determined 30 min post either solvent (control) or Vitex extract i.p. injection. * indicates significant in comparison to the control group ($p<0.05$, n= 6 - 8 per group)

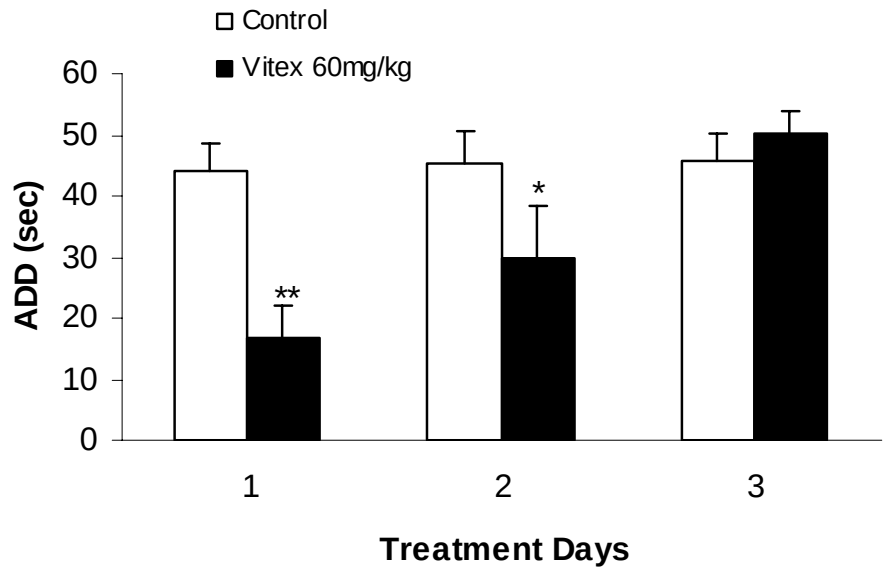

Fig. 2- Vitex treatment reduced afterdischarge duration (ADD as seconds) in amygdala kindling model in comparison to the control group (n=5-7) in male rats. Each group of animals received daily either Vitex or solvent (control) and ADD was determined 30 min post injection (i.p.). * Indicates p<0.05 and **, p<0.01

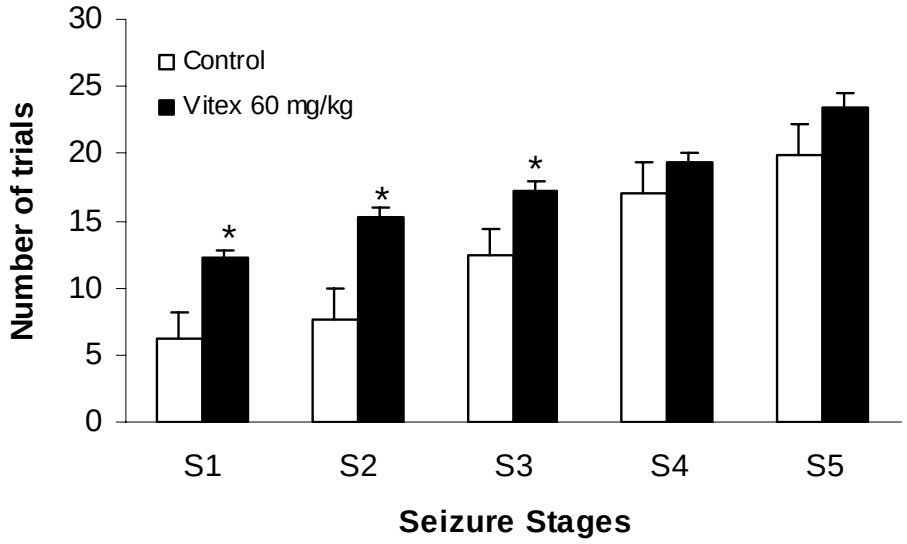

Fig. 3 - Effect of Vitex on number of trials for each seizure stage (S) in electrical amygdala kindling model of epilepsy in intact male rats. The animals stimulated daily with the first AD threshold 30 min post either solvent (control) or Vitex extract injection (i.p.). * indicates significant in comparison to the control group (p<0.05, n= 6 - 8 per group)

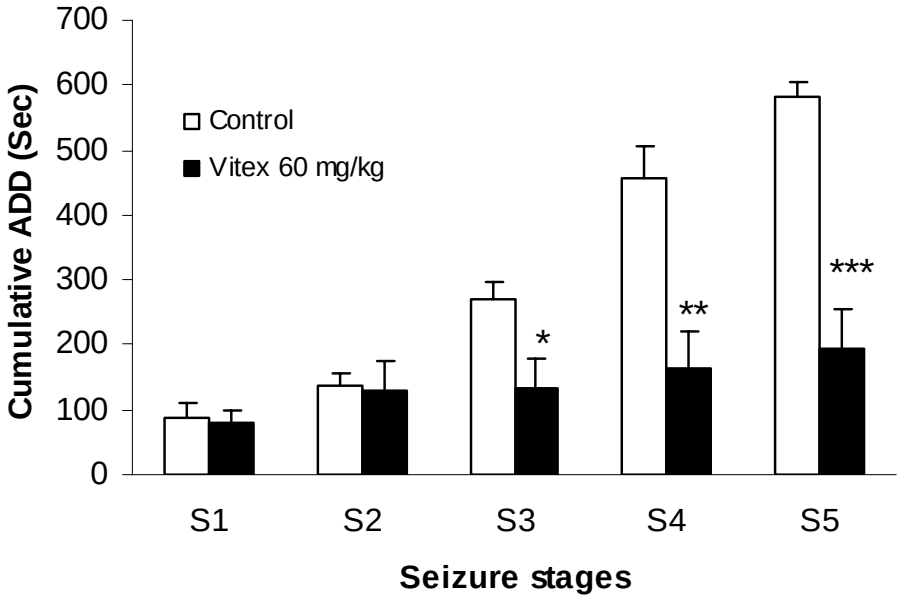

Fig. 4- Effect of Vitex on cumulative ADD to reach each seizure stage (S) during kindling acquisition. The animals received either solvent (control) or Vitex extract and ADD was determined 30 min post injection. * indicates significant in comparison to the control group (p<0.05) **, p<0.01, and ***, p< 0.001 (n= 6 - 7 per group)

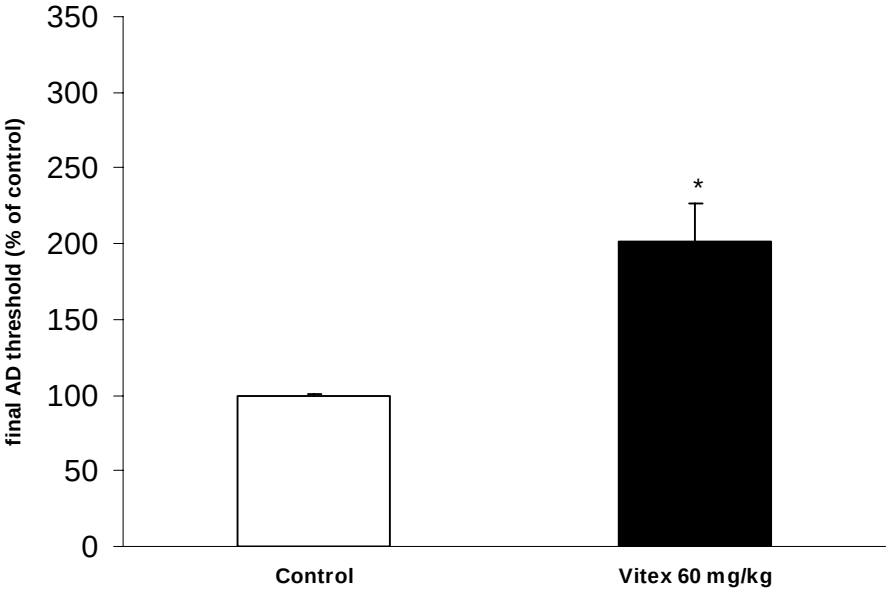

Fig. 5- The mean of final AD threshold after full kindling acquisition. * indicates significant in comparison to the control group (p<0.05)

Discussion

Historically, Vitex had been used with other extracts as "black remedy" for treatment of epilepsy and psychosis in 1200 A.D [3]. The extract of Vitex negundo leaves have shown significant protection against strychnine and pentylenetetrazole (PTZ) induced convulsion [12] and decreased the number and

duration of convulsion in PTZ model, but have not been effective against maximal electroshock seizures (MES) [13]. The electrical kindling model is considered as one of the outstanding investigational models for human complex partial seizures with secondary generalization [14, 15]. This model as an accurate and accepted method was recruited to evaluate anti-epileptic effects of Vitex fruit extract. The present study is the first one which addresses anti-epileptic effect of Vitex fruit extract in electrical kindling model of epilepsy.

The AD threshold increment in Vitex treated intact animals is due to Vitex ability to inhibit neuronal excitability and so evoked seizures at least in the amygdala. The reduction of ADD after treatment with Vitex implies that the extract may inhibit discharge repetition and consequently epileptic activity. This effect is decreased in continuous usage, which may be associated with predominant estrogenic effects in chronic treatment [3, 11, 17]. Recent cell culture experiments have indicated that extracts of Vitex may contain yet unidentified phytoestrogens. Estrogenic actions are mediated via estrogen receptors (ER). These data have demonstrated that the phytoestrogens in Vitex are ERs-selective [11]**.** The estrogenic activity can decrease AD threshold and accelerate kindling acquisition in male rats [17]. So the estrogenic effect of Vitex probably interacts with ADD decrement, kindling acquisition and consequently its antiepileptic activity.

Although the required number of stimulations was not significantly increased for achievement of full kindling, but this effect was significant for S1 to S3. These results demonstrate that Vitex can postpone initial stages (S1 to S3) of kindling acquisition and are in accordance with its effects on ADD and AD threshold. Based on these findings, Vitex possibly retards rapid progression and development of epileptic foci. It should be mentioned that animals in Vitex treated group have responded to higher AD threshold to emerge AD and so, received more potent stimulations in comparison to the control group during kindling acquisition. This difference shows more resistance against kindling during Vitex treatment and is due to Vitex antiepileptic activity.

Chronic usage of the extract reduced significantly the cumulative ADDs from S3 to S5 which is correlated with the ADD reduction. Since, prolonged and additional afterdischarges can lead to more neuronal injuries, seizure progression and prolonged stability; ADD reduction could be considered as an advantage which can interfere with kindling acquisition and stability. In addition, animals which reached to full kindling during Vitex treatment, remitted to S3 or S4 after two weeks free of stimulation period. This effect shows instability of full kindling in Vitex treated animals.

Based on previous studies, Vitex extract has 4 major effects including: 1) Agonistic activity on mu, kappa and delta opioid receptors [8, 9]; 2) Agonistic activity on D2 receptors [9, 10]; 3) Inhibition of the acetylcholine release in a concentration-dependent manner [9]; 4) Estrogenic effects [7, 11]. Yajima and coworkers [19] demonstrated that activation of both mu- and delta-opioid receptors increases the incidence of convulsions produced by blockade of GABA synaptic transmission and stimulation of kappa-opioid receptors has an anticonvulsive effect. In another study subcutaneous or i.c.v. administration of U50, 488 (a highly selective kappa opioid agonist) resulted in a dose-and time-dependent anticonvulsant action in rats [20]. According to these studies, there might be a relationship

between the opioid receptors tendency of Vitex extract and its antiepileptic activity.

On the other hand blockade of D2 receptors in the hippocampus has markedly lowered the seizure threshold to pilocarpine. [21] and D1 agonist such as SKF38393 can cause a dose-dependent blockade of pentylenetetrazole-induced seizures. In addition the anticonvulsant effect of dopamine agonists such as apomorphine appears to be mediated by postsynaptic of both dopamine D1 and D2 receptors [22].

In conclusion, the effect of Vitex on both kappa opioid and D2 dopaminergic receptors may be involved in its antiepileptic activity. Although, mutations in the nicotinic acetylcholine receptors or acetylcholine increment are also responsible for the nocturnal frontal lobe epilepsy [23] and Vitex can decrease acetylcholine release and so, epileptic activity [9].

Vitex also is used for the treatment of premenstrual symptoms. The mechanism of action is proposed to be dopaminergic and estrogenic activities [11]. The linoleic acid from the fruits of Vitex can bind to estrogen receptors and induce certain estrogenic inducible genes [7].

In this study we showed that Vitex extract could act as an antiepileptic agent in amygdale kindled rats. The mechanism of this effect is not apparent but Vitex may achieve this property via four aforementioned mechanisms. Authors encourage further research about effects of chronic administration of Vitex on epilepsy. Also we promote further research for determining the exact mechanisms of Vitex extract antiepileptic activity.

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