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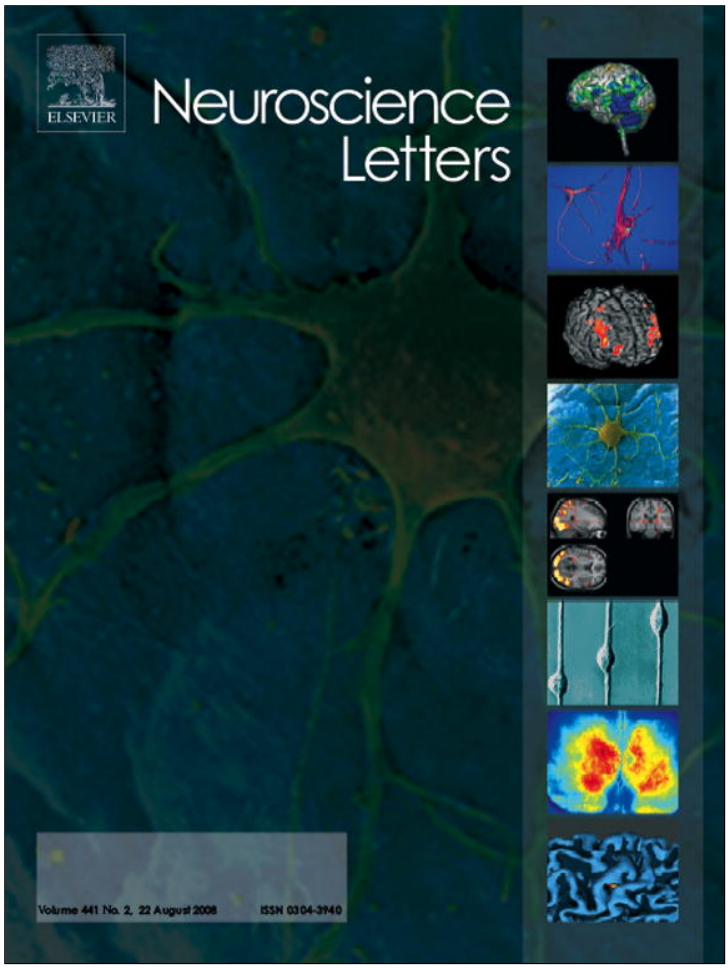

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The antiepileptic activity of *Vitex agnus castus* extract on amygdala kindled seizures in male rats

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ABSTRACT

The antiepileptic activity of hydrophilic extract of *Vitex agnus castus* fruit (*Vitex*) was evaluated by the kindling model of epilepsy. Intact male rats (250–300 g) were stereotaxically implanted with a tripolar and two monopolar electrodes in amygdala and dura, respectively. The afterdischarge (AD) threshold was determined in each animal and stimulated daily until fully kindled. The animals were administered different doses (60, 120 or 180 mg/kg) of *Vitex* or 0.1 ml of hydro alcoholic solvent intra-peritoneally (i.p.) and kindling parameters including AD threshold, seizure stages (SS), afterdischarge duration (ADD), stage 4 latency (S4L) and stage 5 duration (S5D) were recorded 30 min post-injection. The obtained data showed that even low dose (60 mg/kg) of *Vitex* could significantly increase the AD threshold and decrease the ADD and S5D ($P < 0.05$). These changes were more significant with higher doses (120 or 180 mg/kg) for ADD ($P < 0.01$) and S5D ($P < 0.001$). *Vitex* at the dose of 120 mg/kg, induced significant increment in S4L ($P < 0.05$). This effect was more prominent at the dose of 180 mg/kg ($P < 0.001$). The latter dose could significantly reduce seizure stage ($P < 0.01$) and most of the animals did not show S5. These results indicate that *Vitex* can reduce or prevent epileptic activity as demonstrated by reduction of ADD and S5D (length of convulsion) in a dose dependent manner. In conclusion, *Vitex* at appropriate dose can probably reduce or control epileptic activities.

Epilepsy is the second most common neurological disorder after the stroke [15]. In many cases, even multi-drug therapy is not effective and neurosurgical procedures may be indispensable [6]. Thus, research for finding new drugs with less adverse effects and more efficacy, seems to be essential. As many of the herbal drugs have few adverse effects, assessment of herbal medications for their possible antiepileptic activity is worthwhile.

Vitex known as vitagnus or chaste tree is a deciduous shrub and native to Mediterranean Europe and central Asia. This plant had been well known in Iranian ancient medical schools and Alkandi used this plant for the treatment of epilepsy and psychosis in 1200 A.D. He mixed the *Vitex* extract with other extracts of plants and named it Black remedy [5]. Traditionally, *Vitex* fruit extract has been widely used in the treatment of many female disorders, such as menstrual irregularity, premenstrual syndrome and cyclic mastalgia [4,7,10,11,21]. The major compounds of vitex are Casticin, Luteolin, Rotundifuran and Agnuside [9]. In vitro investigations have elucidated that the lipophilic extract act as agonist for mu and kappa opioid and D2 dopaminergic receptors [20,22], while aqueous fraction has more tendency to bind with delta opioid receptors. The aqueous fraction of methanolic extract inhibits the release of acetylcholine in a concentration-dependent manner [19]. This extract has also shown estrogenic effects that may be related to the presence of linoleic acid as an estrogenic compound [9,11]. In experimental model the methanolic extract of *Vitex negundo* leaves have shown significant protection against strychnine and pentylenetetrazole (PTZ)-induced convulsion in mice [8]. In addition, the ethanolic extract of leaves decreased the number and duration of convulsion in PTZ model; however, it had no effect against maximal electroshock seizures (MES) [18]. The electrical kindling model is regarded as one of the most appropriate experimental model because of its similarity to human complex partial seizures [12,16].

Based on these evidences and the traditional usage of this plant as an antiepileptic drug, its reported neuromodulator properties and no report of serious adverse effects [5], in this study the antiepileptic activity of this plant was evaluated, in the electrical kindling model of epilepsy. We investigated the effect of *Vitex* on

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afterdischarge (AD) threshold (after kindling), afterdischarge duration (ADD), stage 4 latency (S4L), stage 5 duration (S5D) and seizure stages (SS) in male rats.

Adult male Albino Wistar rats weighing 250–350 g, were housed at constant temperature (21 ± 2 °C) and 12 h light–dark cycle (lights on 7:00–19:00) and relative humidity (55 ± 20%) on a commercial diet, with tap water available ad libitum. Animals were acclimatized to the laboratory conditions for at least 5 days before experiments.

Hydro alcoholic extract of *Vitex* fruit was purchased from Sina Daru company (Iran).

Under ketamine (50 mg/kg) anesthesia, animals 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 mm 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 earth and differential electrodes, respectively. Ten days after the surgery, electrical kindling was started by determining the AD threshold. Kindling stimulation (2 s, 100 Hz, monophasic square wave pulses of 0.5 ms per half wave) was initially delivered at 10 μA with an ascending series of 10 at 5 min intervals, until at least 5 s of AD was recorded, as previously described [16]. Convulsive responses during kindling were identified using the stages 0–5 paradigms of Racine as follows.

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; stage 5, rearing, forelimb clonus and loss of equilibrium. All animals were kindled to five consecutive stage 5 seizure before receiving any drugs [17].

Fully kindled animals were randomly allocated in one of the different groups ($n = 6–8$ per group), and the AD threshold, seizure stages, ADD, S4L and S5D were determined in each animal as pretreatment control. Then, after 24 h, each group received a single dose of either, solvent of *Vitex* (0.01 ml of hydroalcoholic solution, intra-peritoneally (i.p.)) in control group, 60, 120 or 180 mg/kg of *Vitex*, i.p. and kindling parameters as cited above were reordered. The separate group for control was used to eliminate the effect of repeated stimulations and injections on AD threshold and kindling parameters.

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

The results were expressed as mean ± S.E.M. and statistical significance was evaluated by one way ANOVA. $P < 0.05$ was considered as significant.

Data expressed as percent of stimulation (AD) threshold, were compared within and between groups by Wilcoxon and Mann–Whitney *U*-test, respectively.

All kindled animals responded with stage 5 of seizure, and histological analysis at the end of the procedures verified placement of the stimulating electrode tips at the basolateral amygdala. *Vitex* extract treatment was associated with significantly increased AD threshold in a dose dependent manner (Fig. 1). The AD threshold increased about 3.8 times at the dose of 180 mg/kg ($P < 0.001$) in comparison to the control group. Administration of the different doses of *Vitex* extract caused almost similar effects on kindling parameters, including significant reduction of ADD (Fig. 2), and S5D (Fig. 3) and S4L prolongation (Fig. 4) in a dose dependent manner. *Vitex* at the dose of 120 mg/kg, induced significant increment in S4L ($P < 0.05$). This effect was more prominent with the dose of 180 mg/kg ($P < 0.001$). The dose of 180 mg/kg changed markedly SS in fully kindled animals and most of the animals did not show S5 or seizure development, remained unresponsive or showed only S2 and S3 after stimulation (Fig. 5).

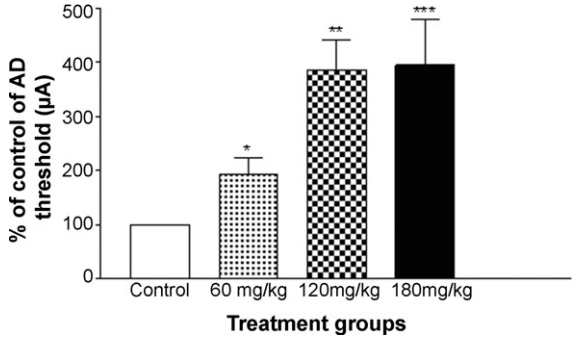

Fig. 1. Effect of *Vitex agnus castus* on AD threshold in electrical amygdala kindling model of epilepsy in male rats. The AD threshold was determined 30 min post-either solvent (control) or Vitex extract i.p. injection. Significant in comparison to the control group $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ ($n = 6–7$ per group).

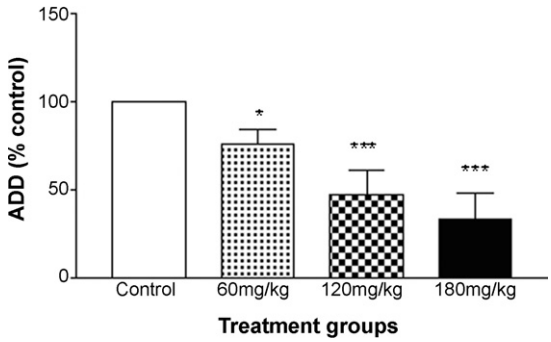

Fig. 2. *Vitex* treatment reduced afterdischarge duration (ADD as seconds) in amygdala kindled seizure in comparison to the control group ($n = 5–7$) in male rats. Each group of animals received daily either *Vitex* or solvent (control) and ADD was determined 30 min post-injection (i.p.). Significant in comparison to the control group $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ ($n = 6–7$ per group).

The electrical kindling model of epilepsy is the most appropriate investigational model to study human complex partial seizures with secondary generalization [13,17]. This model as an accurate, quantitative and accepted method was selected to evaluate antiepileptic effects of *Vitex* fruit extract.

Historically, the mixture of *Vitex* with other extracts had been used as "black remedy" for treatment of epilepsy [5]. However, there is not any published data regarding effects of *Vitex* extracts on epilepsy. This study was designed to evaluate antiepileptic effect of *Vitex* fruit extract in kindling model of epilepsy.

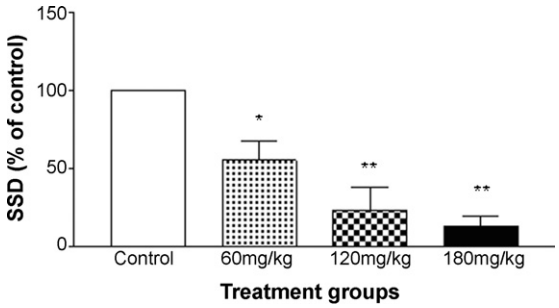

Fig. 3. *Vitex* treatment reduced stage 5 duration (S5D as seconds) in amygdala kindled seizure in comparison to the control group ($n = 5–7$) in male rats. Each group of animals received daily either *Vitex* or solvent (control) and ADD was determined 30 min post-injection (i.p.). Significant in comparison to the control group $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ ($n = 6–7$ per group).

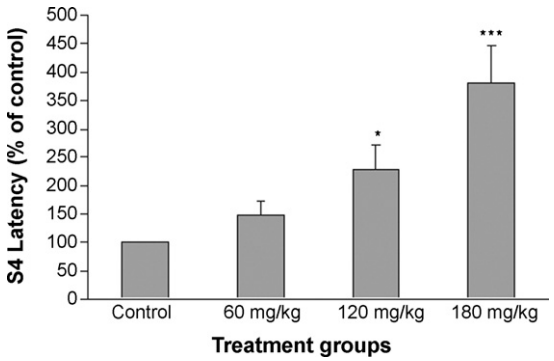

Fig. 4. Effect of *Vitex agnus castus* on S4 latency (S4L) in amygdala kindled seizures in male rats. The animals stimulated 30 min post-either solvent (control) or Vitex extract i.p. injection and S4L was determined during recording. Significant in comparison to the control group $^{*}P < 0.05$ and $^{***}P < 0.001$ ($n = 6$–7 per group).

The AD threshold increment in kindled animals after treatment with *Vitex* extract is due to the ability of *Vitex* to inhibit neuronal excitability at least in the amygdala. This effect may prevent or delay evoked seizures. However, further investigation is needed to clarify whether this effect is caused by reduction of resting membrane potential or by an increase in neuronal stimulation threshold at cellular level. In agreement with our results, the methanolic extract of *Vitex negundo* leaves (another species of *Vitex*) have shown significant protection against strychnine and PTZ-induced convulsion in mice [8] and decreased the number and duration of convulsion in PTZ model [19].

Vitex-induced ADD reduction is an indication of reduced repeated firing and excitability of neuronal tissues at least in the amygdala. So, it may inhibit discharge repetition and thus reduce epileptic activity. The S4L prolongation is a valuable index showing a more delay from stimulation to generalized seizures and indicates that *Vitex* could postpone AD distribution from a nucleus such as amygdala to other regions of the brain including cortex.

The S5D reduction or inhibition (at high dose of *Vitex*) is a strong evidence for antiepileptic or seizure activity of *Vitex*. This effect was shown for *Vitex Negundo* leaves in PTZ or strychnine models of epilepsy [8].

Vitex extract has agonistic activity on mu, kappa and delta opioid receptors [23,24]. Previous studies have suggested that opioids have a protective role against seizure activity in the gerbil and the opioid anticonvulsant effect is not specific to one type of opioid agonist [11]. It has been shown that stimulation of kappa opioid receptors could induce an anticonvulsive effect [25] and 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]. These studies, thus suggest that there is a relationship between the opioid receptor activities or agonistic properties of *Vitex* extract and its anticonvulsant activity.

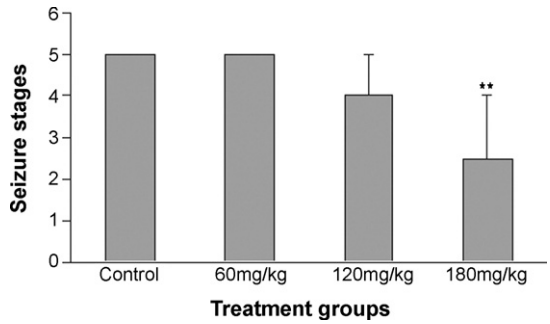

Fig. 5. Effect of *Vitex agnus castus* on seizure stage (SS) in amygdala kindled seizures in male rats. The animals stimulated 30 min post-either solvent (control) or *Vitex* extract i.p. injection and the SS was determined during recording. Significant in comparison to the control group, $^{**}P < 0.01$, and ($n = 6$–7 per group).

While blockade of D2 receptors in the hippocampus has markedly lowered the seizure threshold to pilocarpine [1], but D2 agonists such as quinpirole only weakly blocks the action of PTZ [2]. On the other hand, a D1 agonist like SKF 38393 causes a dose-dependent blockade of PTZ-induced seizures. In addition, the anticonvulsant effect of dopamine agonists such as apomorphine appears to be mediated by postsynaptic activities of both dopamine D1 and D2 receptors [14].

It seems that, both kappa opioid and D2 dopaminergic activities of *Vitex* may be involved in *Vitex* anticonvulsant activity. A further investigation with a selective kappa receptor antagonist or D2 receptor agonist is warranted to elucidate the role these receptors, or neurotransmitters in *Vitex* antiepileptic activity. Mutations in the nicotinic acetylcholine receptors or acetylcholine increment are also responsible for the nocturnal frontal lobe epilepsy [3]. Decrease in acetylcholine release by *Vitex* thus can be additional mechanism for its anticonvulsant activity [19].

In this study we showed that *Vitex* extract could act as an antiepileptic agent in amygdale kindled rats. The exact mechanism of this effect is not clear however *Vitex* may achieve it via several neurotransmitter systems. Further studies on its chronic effects as well as its exact mechanism of actions are required.

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