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Study of the effect of *Ocimum basilicum* Hydro-Alcoholic Extract on Convulsion Induced by Nicotine in Mice.

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ABSTRACT

Convulsion is defined as the abnormal electrical discharge of a group of brain neurons causing a temporary disorder in the normal activity of brain. Basil (*Ocimum basilicum*) is an herb with remedial effects cited in traditional medicine. This study evaluates the effect of basil hydro alcoholic extract on nicotine-induced convulsion. Nicotine -induced convulsion in mice was used as the animal model. Mice was administered nicotine (5 mg/kg,ip) and hydro alcoholic extract of Ocimum basilicum(400, 800, 1200& 1600mg/kg, ip, respectively). Then, latency, duration and intensity of convulsion were studied. When the most effective dose of basil was determined, the extract was injected 15, 30, 45 & 60 min before receiving nicotine in order to find the best time of injection. Basil hydroalcoholic extract with a dose of 800, 1200, and 1600 mg/kg has a significant effect on convulsion factors. The highest anti-convulsion effect was observed in dose of 1200 mg/kg. The best result was obtained when this dose of basil was injected 30 minutes before injecting nicotine. Basil hydro alcoholic extract affect Latency, duration, and intensity of convulsion.

Keywords: Ocimum basilicum, Nicotine, Diazepam, Convulsion, Mice

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INTRODUCTION

Epilepsy is considered as repeated convulsion attacks caused by genetic diseases or brain damages [1]. Its prevalence in the world is 1% and is known as the second cause of central nervous system diseases [2].

Convulsion occurs due to the over-activity of brain neurons and is defined as periods of neurological abnormal activity [2]. Convulsion attacks have different causes ranging from different nervous diseases to infection, tumor, brain trauma, congenital diseases, fever, and toxic and metabolic factors [1]. The key point in the treatment of epilepsy is its long-term treatment which underlies the occurrence of different side-effects of related drugs.

Anti-convulsant drugs may be associated with different side-effects such as liver damage, blood disorders, memory attenuation, fatigue, and sleepiness, which restrict the application of these drugs. Therefore, research on this filed has been continued to find more effective anti-epileptic drugs. Traditional medicine and herbs are the proper sources of such drugs. Herbal tranquilizers may have the same potential of anti-convulsion drugs [1].

Basil (*Ocimum basilicum*) is an herb of Lamiaceae family. It has a straight and conic root with a length of 10-16 cm and numerous branches. Its stem is straight with rectangular section with more or less branches. It grows in different heights ranging from 40-60 cm depending on the climate in which it grows. Basil has wide leaves positioned opposite to the stem with green color and flat edges. It has pink or white light color, small flowers integrated in the form of 6-flower limbs located at the end of the main and subsidiary stems [6]. Its origin is India, Iran, and Afghanistan.

As an herb, basil has been used from ancient times to treat different diseases like anxiety, worry, insomnia, migraine attacks, and stress [8]. Phenylpropanoids are the main and important ingredient of basil accounting for 90% of its essence. Eugenol, chavicol, mercotin, phenyl cinnamate and elmicin are the most important ingredients of basil that give it remedial effects [9].

S. Olivera et al proved in a study that the volatile oil of basil controlled the pentylenetetrazol- and picrotoxin-induced convulsion [10]. The present study tries to evaluate the protective effect of basil on nicotine-induced convulsion.

METHODOLOGY

Preparation of the extract

Basil (*Ocimum basilicum*) leaves were obtained and dried in a dark place at laboratory temperature. Then, the dried leaves were grounded by an electric mill and were kept in refrigerator within a sealed container. After weighing, the powder was poured to an appropriate container and 70% ethanol was added. The container lid was closed and kept for 72 hours. Within the 72 hours, the contents of the container were mixed every 12 hours. After 72 hours, the solution was filtered through filter paper and was concentrated in a vacuum distillation unit. The concentrated extract was dried in an oven at 40 °C.

Laboratory animals

The N.Mari mice was obtained from the animal house of Jundishapour University of Medical Sciences, Ahvaz, Iran, and kept under specific conditions, on a constant 12-hour light/dark cycle, at a controlled temperature of 23 ± 2°C. Standard pellet food and tap water were available ad labitum.. The mouse then randomly divided into nine equal groups (n = 8); a positive and negative control group and four study groups. The test groups received hydroalcoholic extract of Ocimum basilicum plus nicotine respectively. The negetive control group received normal saline (10 mg/kg, ip) and positive control group received diazepam (0.15 mg/kg,ip)

Drugs

Nicotine with a dose of 5 mg/kg was used to induce convulsion. *Investigation of the extract effect on nicotine-induced convulsion*

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Dose-response study

In order to investigate the anti-convulsion effect of basil, the mice were randomly divided into groups of eight. Basil with a dose of 400, 800, 1200, and 1600 mg/kg was injected to the mice. The negative control group received 10 mg/kg normal saline while positive control group received 0.15 mg/kg diazepam. All groups received 5 mg/kg nicotine after half an hour. After injecting nicotine, the mice were immediately transferred to a separate cage and convulsion initiation, convulsion duration, and convulsion intensity as well as the mortality rate were studied.

In order to measure the intensity of convulsion, the mice were placed on a desk. If they showed normal actions they were scored zero, if their head shook slightly they were scored 1, if their head and jaw shook severely they were scored 2, if their body shook slightly they were scored 3, and if their entire body shook severely they were scored 4.

Time-response study

Following the dose-response study, the best obtained dose was injected 15, 30, 45, and 60 minutes before injecting nicotine. During the test, convulsion initiation, convulsion duration, convulsion intensity, and mortality rate were evaluated.

Statistical Analysis:

The data are expressed as mean ± SE. Statistical differences between means were determined by one-way analysis of variance (ANOVA), followed by LSD test and a threshold of significance of $P < 0.05$.

RESULTS

Dose-Response

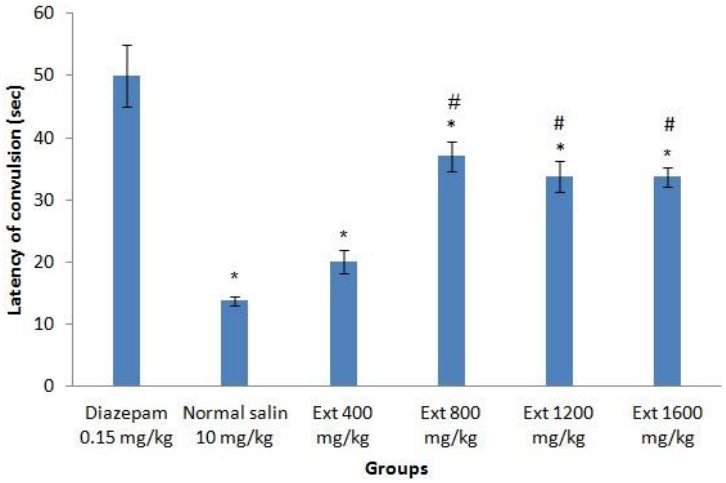

Figure 1: Comparison of the effects of different doses of basil hydroalcoholic extract on latency of convulsion between the positive control group received 0.15 mg/kg diazepam and the negative control group received 10 mg/kg normal saline.

* The difference with diazepam receiving group (0.15 mg/kg) is significant (p<0.05).
# The difference with normal saline receiving group (10 mg/kg) is significant (p<0.05).
N=8

Figure 1 shows that basil hydoalcoholic extract with a dose of 800, 1200, and 1600 mg/kg significantly increases onset of convulsion compared with the control group (p<0.05), while in dose of 400 mg/kg there was no significant difference with the control group.

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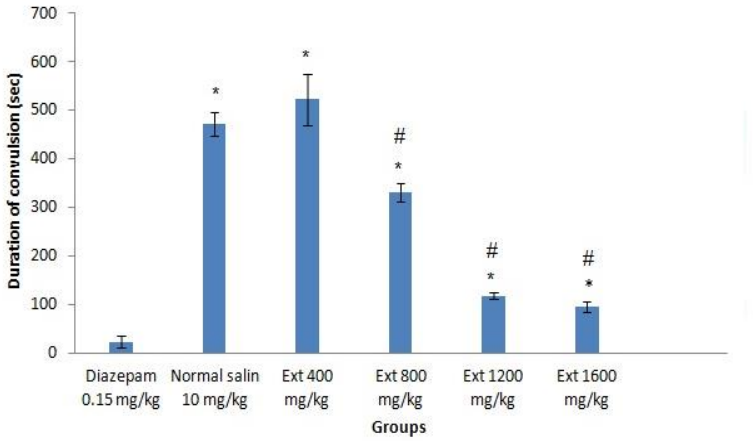

Figure 2: Comparison of the effects of different doses of basil hydroalcoholic extract on duration of convulsion between the positive control group received 0.15 mg/kg diazepam and the negative control group received 10 mg/kg normal saline.

* The difference with diazepam receiving group (0.15 mg/kg) is significant (p<0.05).
# The difference with normal saline receiving group (10 mg/kg) is significant (p<0.05).
N=8

Figure 2 shows that basil hydro alcoholic extract with a dose of 800, 1200, and 1600 mg/kg significantly controls duration of convulsion compared with the control group (p<0.05), while in dose of 400 mg/kg there was no significant difference with the control group.

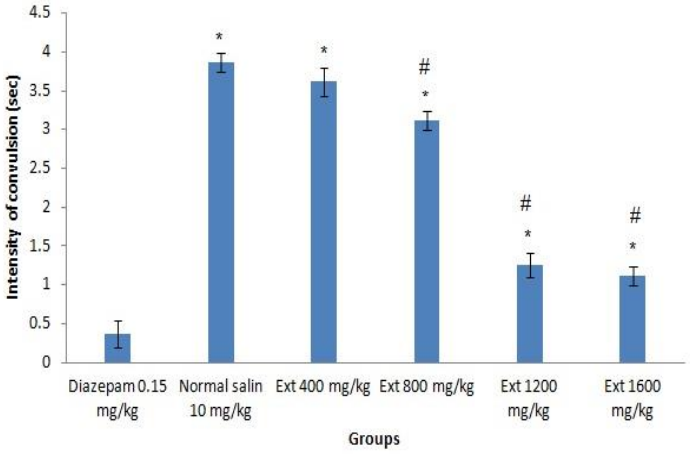

Figure 3: Comparison of the effects of different doses of basil hydroalcoholic extract on intensity of convulsion between the positive control group received 0.15 mg/kg diazepam and the negative control group received 10 mg/kg normal saline.

* The difference with diazepam receiving group (0.15 mg/kg) is significant (p<0.05).
# The difference with normal saline receiving group (10 mg/kg) is significant (p<0.05).
N=8

Figure 3 shows that basil hydro alcoholic extract with a dose of 800, 1200, and 1600 mg/kg significantly controls intensity of convulsion compared with the control group (p<0.05), while in dose of 400 mg/kg there was no significant difference with the control group.

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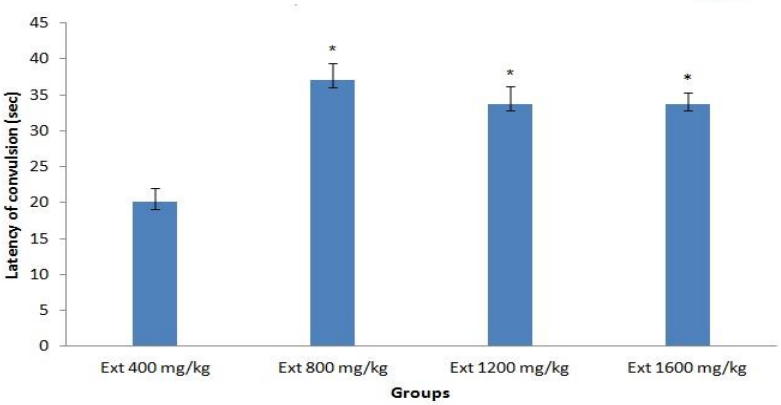

Figure 4: comparison of the effects of different doses of hydroalcoholic extract on latency of convulsion The difference with group receiving dose of 400 mg/kg is significant (p<0.05).*

N=8

Figure 4 shows that basil hydoalcoholic extract with a dose of 800, 1200, and 1600 mg/kg significantly controls intensity of convulsion compared with the group receiving dose of 400 mg/kg(p<0.05)

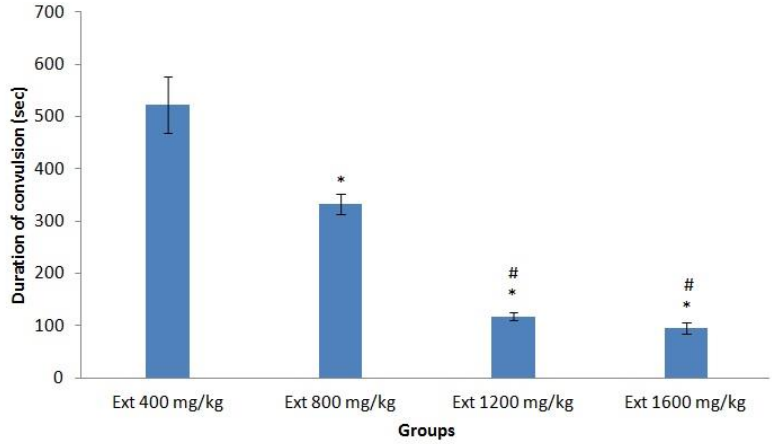

Figure 5: comparison of different doses of hydroalcoholic extract on duration of convulsion

The difference with group receiving dose of 400 mg/kg is significant (p<0.05).*
The difference with the group receiving dose of 800 mg/kg is significant (p<0.05)#
N=8

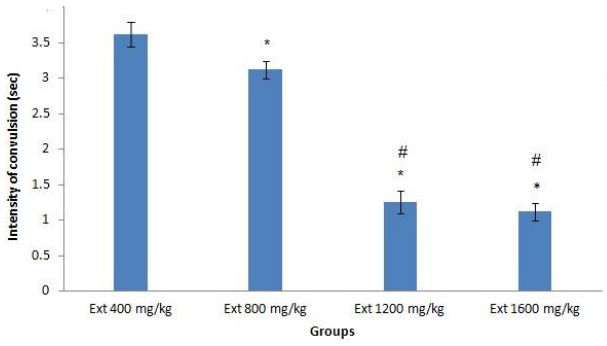

Figure 6: comparison of differen doses of hydroalcoholic extract on of convulsion

The difference with group receiving dose of 400 mg/kg is significant (p<0.05).*
The difference with the group receiving dose of 800 mg/kg is significant (p<0.05)#
N=8

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Figure 5 shows that basil hydoalcoholic extract with a dose of 800, 1200, and 1600 mg/kg significantly controls intensity of convulsion compared with the group receiving dose of 400 mg/kg(p<0.05)and groups receiving dose of 1200 & 1600 mg/kg shows significant difference with dose of 800 mg/kg. p<0.05 . there was no significant difference between doses of 1200 & 1600 mg/kg.

Figure 6 shows that basil hydro alcoholic extract with a dose of 800, 1200, and 1600 mg/kg significantly controls intensity of convulsion compared with the group receiving dose of 400 mg/kg(p<0.05)and groups receiving dose of 1200 & 1600 mg/kg shows significant difference with dose of 800 mg/kg. p<0.05 . there was no significant difference between doses of 1200 & 1600 mg/kg

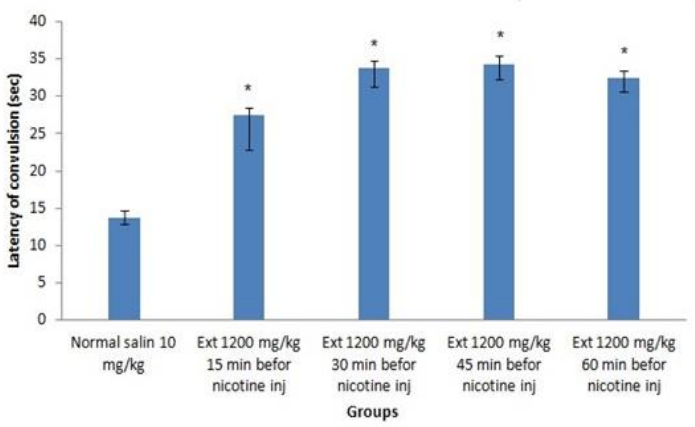

Figure 7: comparison of groups receiving dose 1200 mg/kg of hydroalcoholic extract 15,30.45 & 60 min before injecting nicotine with normal salin on latency of convulsion

*The difference with normal saline receiving group (10 mg/kg) is significant (p<0.05).
N=8

Figure7 shows effects of the best dose (1200 mg/kg) in different times before injecting nicotine in comparison with normal salin .All groups increase onset of convulsion compared with control groups.p<0.05

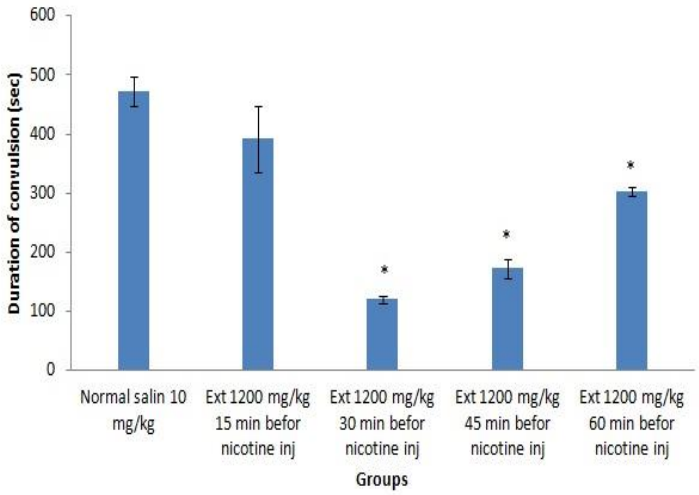

Figure 8: comparison of groups receiving dose 1200 mg/kg of hydroalcoholic extract 15,30.45 & 60 min before injecting nicotine with normal salin on duration of convulsion

*The difference with normal saline receiving group (10 mg/kg) is significant (p<0.05).
N=8

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Figure 8 shows effects of the best dose (1200 mg/kg) in different times before injecting nicotine on latency of convulsion in comparison with normal salin .All groups control duration of convulsion compared with control groups.p<0.05

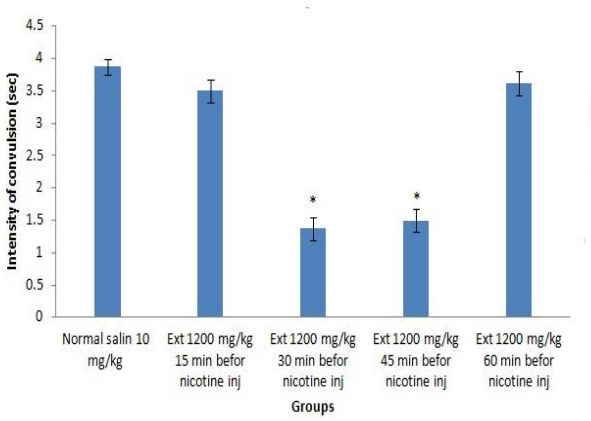

Figure 9: comparison of groups receiving dose 1200 mg/kg of hydroalcoholic extract 15 ,30, 45 & 60 min before injecting nicotine with normal salin on latency of convulsion

N=8

*The difference with normal saline receiving group (10 mg/kg) is significant (p<0.05).

Figure9 shows effects of the best dose (1200 mg/kg) in different times before injecting nicotine on latency of convulsion in comparison with normal salin .All groups delayed inition of convulsion compared with control groups. p<0.05

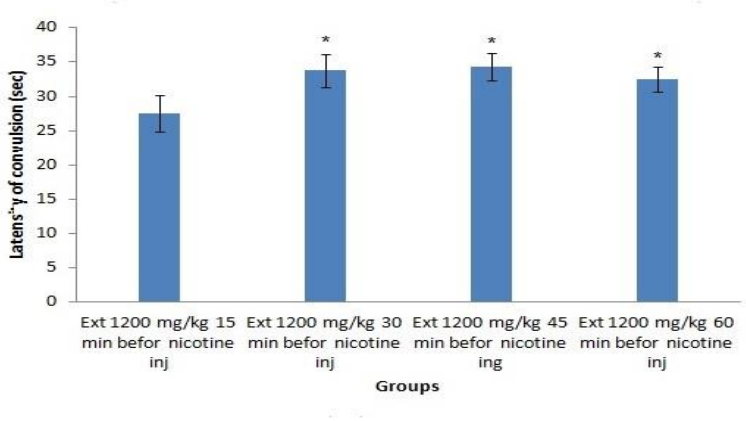

Figure 10: comparison of latency of convulsion in groups receiving dose of 1200 mg/kg 15, 30,45 & 60 min before injecting nicotine

*The difference with groups receiving dose of 1200 mg/kg 15 min before nicotine injecting) is significant (p<0.05)

N=8

Figure 10 shows groups receiving the best dose (1200 mg/kg) in 30, 45 & 60 min before injecting nicotine significantly increases onset of convulsion in comparison with groups receiving dose of 1200 mg/kg 15 min before injecting nicotine.p<0.05

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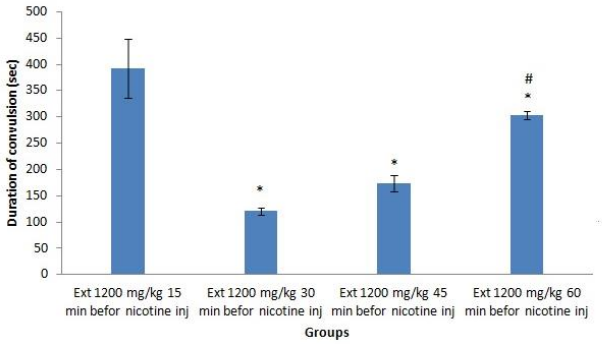

Figure 11: comparison of duration of convulsion in groups receiving dose of 1200 mg/kg 15, 30,45 & 60 min before injecting nicotine

*The difference with groups receiving dose of 1200 mg/kg 15 min before nicotine injecting) is significant (p<0.05).
#The difference with groups receiving dose of 1200 mg/kg 30 & 45 min before nicotine injecting is significant.p<0.05

Figure 11 shows groups receiving the best dose (1200 mg/kg) in 30, 45 & 60 min before injecting nicotine significantly controls duration of convulsion in comparison with groups receiving dose of 1200 mg/kg 15 min before injecting nicotine.p<0.05.groups receiving hydroalcoholic extract 30 & 45 min before injecting nicotine significantly controles duration of convulsion in comparison with group receiving the dose of hydroalcoholic extract 60 min before nicotine injecting.

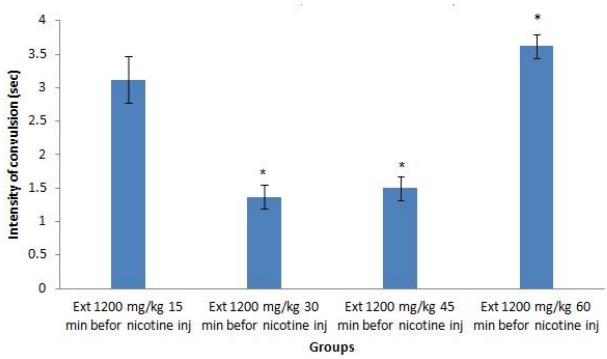

Figure 12: comparison of intensity of convulsion in groups receiving dose of 1200 mg/kg 15, 30,45 & 60 mian before injecting nicotine

*The difference with groups receiving dose of 1200 mg/kg 15 min before nicotine injecting) is significant (p<0.05).
#The difference with groups receiving dose of 1200 mg/kg 30 & 45 min before nicotine injecting is significant.p<0.05

Figure 12 shows groups receiving the best dose (1200 mg/kg) in 30, 45 & 60 min before injecting nicotine significantly controls intensity of convulsion in comparison with groups receiving dose of 1200 mg/kg 15 min before injecting nicotine.p<0.05. groups receiving hydroalcoholic extract 30 & 45 min before injecting nicotine significantly controls intensity of convulsion in comparison with group receiving the dose of hydroalcoholic extract 60 min before nicotine injecting.

DISSCUSSION

As medicinal compounds with low side-effects, herbs have attracted attentions from ancient times. They have been used as proper alternatives in the treatment of different diseases. [11].

Herbs have had medicinal applications from many centuries ago and their remedial and non-harmful effects have been proved during many years. This is why research has been attracted toward traditional medicine and herbs for treatment/control of different diseases. Another reason that highlights herbs is the fact that besides the main ingredient of the plants, they have other effective substances that magnify their remedial effects.

There are different models with their unique mechanism causing convulsion. With a dose of 5 mg/kg, nicotine may trigger convulsion. Studies have indicated the relation between nicotine-induced convulsion and the activation of nicotine receptors in the brain. The convulsion-inducing effect of nicotine is likely backs to its controlling effect on the controlling spinal neurotransmitters *i.e.* glycine or its excitatory effect on psychomotor [12].

Eugenol in basil can control voltage-dependent sodium channels, can control glutamate receptor, and can strengthen GABA effect on GABAergic receptor [13, 14]. The study of Modarressi on the basil hydroalcohoic effect on pentylenetetrazole-induced convulsion confirmed the above results. Despite the findings, conducting more studies aimed at finding mechanism by which basil can prevent nicotine-induced convulsion seems necessary.

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